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**A Tribute to William R. Branch
(1946–2018)**

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Cover photo legend, in Bill's own words:

This remains one of my favourite shots, although it is an old slide and this is the best digital scan I have of it (but of woefully low res). It was taken about 25+ years ago, and I was driving to work when I saw these flowers in bloom beside Port Elizabeth airport. I had an image of a cobra rearing in front of them, the Port Elizabeth Snake Park had just got a beautiful Cape Cobra in from the Northern Cape, and so I asked Rob Hall to come and help manipulate the snake. I didn't have a long lens and so had to lie on my belly with a 55mm Nikon with 1.4 convertor. I used in-fill flash, held by Rob about 1m away and to soften the deep shadow under the snake's belly. I kept shuffling forward to get a more dramatic pose and had taken several shots when the snake disappeared from the viewfinder. Rob was standing to the side holding the flash and also a snake stick to ward off the cobra. When the snake disappeared I instinctively rolled back, heard Rob shout "Shit, that was fast!", and the snake bit the camera body about 6cm from my shutter finger. A bead of venom glistened on the camera body. Looking through the lens I had lost all sense of distance and simply got too close to the snake. It remains the closest I've come to a snakebite. Technically the picture works because the snake is alert but its mouth is shut and it is not looking straight at the camera. It therefore doesn't appear too threatening, allowing viewers to admire what remains my favourite snake. Bill Branch



Compilation of personal tributes to William Roy Branch (1946–2018): a loving husband and father, a good friend, and a mentor

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Abstract.—Personal contributions to William “Bill” Roy Branch by family members and colleagues: Colin Tilbury, Alan Channing, Dot Hall (Pitman, Basson), Rick Shine, James B. Murphy, Luke Verburgt, Julian Bayliss, Michael F. Bates, Pedro Vaz Pinto, Kirsty Kyle, Krystal Tolley, Mzi Mahola, Brian J. Huntley, Roger Bills, Johan Marais, Mark-Oliver Rödel, Paul H. Skelton, Aaron M. Bauer, Stephen Spawls, Andrew Turner, Ernst H.W. Baard, Amber Jackson, Margaretha Hofmeyr, Jens Reissig, Harold Braack, Atherton de Villiers, Marius Burger, Mike Raath, Werner Conradie, and Martin J. Whiting.

Keywords. Influence, contributions, farewell, African herpetology, history, researcher

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On 14 October 2018, William Roy Branch, or simply Bill as he was known to most, passed away after a short struggle with motor neuron disease. He was not only one of South Africa’s most well-known and respected herpetologists, but also a dedicated husband, a father, a good friend, and a mentor to so many of us. We have taken this opportunity to collate personal tributes from family, friends, and colleagues, to showcase the influence Bill had on our lives and careers.

Tributes from family members

Donvé Branch (Bill’s wife)

Bill was an amazing man with a huge passion for life. When we married I introduced Bill to the world of pots and potters, and he introduced me to the world of reptiles and herpetologists. Very different worlds, but they became one we both loved. Over the years I was privileged to meet and host many of you. If I sometimes looked stunned when you arrived at our door, please forgive me. Bill very often failed to tell me we would be having a guest. Together we started to collect art, succulents, and books. None of which we could afford,

but we couldn’t resist.

Bill was a family man who loved and was so proud of his three sons. When we married his generous heart took on my children and grandchildren with the same warmth. Science was his passion which he loved to share. Bill in lecture mode could not be halted. His sense of humour was legendary. A kind, gentle man but also a humble man. He never boasted of his achievements. In his later years, these qualities made him so popular with National Geographic travellers.

A man of huge intellect with a broad knowledge of all things. A kind, generous, and wonderful man. Truly a real mensch. I was so proud to be his wife. He is greatly missed.

James Vlok (Bill’s stepson)

Bill Branch was a man of passion for his craft and natural science. He was an adventurer and an explorer; a man who inspired motivation and discovery of the world around us. He could keep you interested with a keen knowledge and a sense of humour that would have you laughing and learning. He will be sorely missed by family and colleagues alike.

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Christian Vlok (Donvé's grandson)

Gumps told me so many interesting stories about his trips. He gave me my first Masai machete and a lizard. I knew I could ask him anything and he would know the answer. I will miss my Grandpa Gumps so much.

Analeah Vlok (Donvé's granddaughter)

I loved Gumps because he taught me so many things. He taught me which plants I can or can't eat, and about snakes and frogs, which I love. I miss him and every time I go into his room I think of him.

Jenny Vlok (stepdaughter-in-law)

Bill, to look at all things herpetological on a daily basis and know that I can't ask you any more questions about it, fills my heart with such sadness. You were so patient in your explanations, always interesting and funny. With your mismatched socks and wild hair, your fancy salads and poor man's capers, hilarious Easter egg hunts with a difference, cheeky Halloween surprises and Christmas gifting, not only were you an Amazing scientist but also an inspiration, and a motivator, allowing my children to be knowledge bearers and researchers in their own environment. We love you Dear Bill, and will miss you always.

Nicole Kingston (Bill's stepdaughter)

Bill was a rock, a voice of reason, and a safe place and so loved. I am so truly privileged to have known him, and am a better person for it. His kindness, empathy, and wicked sense of humour will not be forgotten.

Oliver Kingston (Donvé's grandson)

Grandpa made me laugh lots and if you wanted to know anything he was the person to ask.

Will Kingston (Donvé's grandson)

He was kind and knew a lot about snakes.

Tributes from friends and colleagues

Colin Tilbury

KwaZulu-Natal, South Africa

It was early in 1980. After a year as a junior medical officer at Ngwelezana Hospital in KZN [KwaZulu-Natal], I had collected a series of cases of snakebites from *Atractaspis bibronii* and the Mozambique Spitting Cobra. With the data in hand I had approached Alan Channing, the then-chairman of the Herpetological Association of Africa, for comments. Being more of the toad persuasion, Alan suggested that I contact his colleague Bill Branch, the incumbent curator of herpetology at the PE [Port Elizabeth] Museum, who had shown more than just a passing interest in snakes and snakebite, and might be in

a better position to help me.

I wrote to Bill and offered to assist with any affairs of the herpetological kind from Zululand. Bill wrote back immediately, expressing a keen interest in the snakebite data and also wondering if I might be able to collect some of the local *Pelusios* for karyotyping. We met for the first time a few months later. Bill was visiting Durban, and Sarah and I arranged to meet him at the British Middle East Indian Sporting and Diners' Club near the Greyville race course, to sample the local curries. A truly memorable evening (I still have intermittent diarrhoea). I think that I may also have introduced Bill to the pleasures of a good red wine—or was it vice versa?

And so began a friendship which lasted nearly 40 years.

Driven with a boundless energy and an amazing zest for life, sharp wit, wry humour, and capacity for sharing, Bill attracted people to him. Whether by active involvement or by association, he had a lasting impact on all those who encountered him. Bill adored the simple things in life, and lived his life simply. He loved the camping and field trips that were an integral part of his work and which provided him with so much satisfaction. An avid angler since childhood, he had pulled many a carp from the rivers and dams of the Eastern Cape. Bill's laboratory and office in the Port Elizabeth Museum was always a wondrous place to visit. Beyond the entrance door which was plastered with a selection of humorous "Bill" references, a mixture of chaos and creativity, preserved snakes and lizards in piles, the air reeking with alcohol, and Bill smiling happily. Bill and Donvé's lovely home in Port Elizabeth was in many ways an extension of his beloved office at the museum. Of the many enduring mental images that capture Bill's essence for me, are none more so than those of Bill at work in his man-cave at home. More like a 'control room,' his desk surmounted with massive computer screens and surrounded on all sides—floor to ceiling—with books, paintings, and photographs (including his all-time favourite of the yellow Cape Cobra that had nearly bitten him). Shelves were packed with w.i.p. files and books with titles covering an eclectic array of topics from tadpoles to volcanoes, fossils, sunbirds, euphorbias, mesembs, and every conceivable reptile and amphibian genre.

At home, but outside his study, every nook and cranny was adorned with paintings and Donvé's beautiful pottery. Each windowsill in the house was crammed with weirdly-shaped, rare, and spiky plants. Their garden was an indigenous plant paradise with a few thorny exotics, a haven for birds and local wildlife where the largest *Palystes* rain spiders in the world were free to roam—although strangely I only ever saw them on the walls of the guest bedroom. Theirs was clearly a home they loved to live in and was always open to the many guests who might pop in and stay over.

One could not know Bill and be unimpressed with his amazing intellect. Bill read—no—he devoured books by the ton. I have never met anyone who had such a command and broad understanding of natural history. He could have been anything from botanist, ornithologist, entomologist, mammologist, geologist, physicist—you name it. The reality in fact, is that he was all of these things and many more; such was the breadth and depth of his knowledge. His intimate understanding of the intricacies of natural history, the environment, and the interconnected webs of life, filling in the dots on life’s canvas one by one—or in Bill’s case, by the dozens at a time.

In spite of his huge talents, he kept his feet firmly on the ground and freely shared his knowledge and wisdom with anyone who asked for advice or input. He was an inspirational force to anyone and everyone who had the privilege to know or work with him; a truly benevolent gentle giant and an incredibly productive scientist. The herpetological community around him was so privileged to have him as a guide and mentor. In the decade following his retirement from the museum, he worked as a specialist guide for over 50 National Geographic touring parties. These afforded him opportunities to continue to pursue herps in many iconic African locations.

As a friend, Bill was caring, insightful, non-judgmental, and always with a wonderful sense of humour just bubbling beneath the surface. As a storyteller he had few peers: in his clipped British accent with the hint of a lisp mumble and a wry smile, he would gleefully extol the excruciating agony of the many unfortunates who became the subjects of his tales. Of course, these often involved his hapless colleagues on the many field trips that he made. Quick, dry, wicked, invariably veiled in intrigue, he would construct the twists and turns of his story to extract every molecule of humour. His punch lines always immaculate.

Over the years, I spent a great deal of time outside the borders of South Africa, but Bill always found time to write and give updates on his projects and movements.

After the birth of our first child in London in July 1989, Sarah and I sent out a short notice of his birth to a few friends and relatives, making reference to ‘*the discovery of a new species of the TILBURY genus found lurking in the St Helier’s Labour Ward at precisely 03h45 hours on 25 July 1989. It is wriggly, pink all over, devoid of scales and tail, and makes characteristic feeding cries every 4 hours. It weighed 3.63 kg on discovery, and has the features characteristic of the male sex. It has been named Douglas Matthew.*’

I left London a week after the event and headed back to my job in Saudi Arabia. Shortly after my return to Khamis Mushayt, I received a letter from Bill:

*“Dear Colin,
Congratulations on the arrival of Douglas Matthew.
You must be looking forward to Sarah and DMT*

arriving at the end of the month, although I suppose that throwing all the Cerastes out to make way for the cot must be a bind. He will slow your globe-trotting down a bit, but it will only be about 12 years before he is useful in the field! Robbie and Matthew do all the hard work in the field now, so they do have their advantages.

I feel I must take exception to the new name however. Looking through the London telephone directory I came across three other references to Tilbury Douglas Matthew (usually from the poorer eastern suburbs besides the Thames). All had priority, some dating from the early 1930’s. Your new name is thus pre-occupied, and according to strict nomenclatural rules (Int. Rules Zool. Nomenclature, rev. ed. London 1986; page 25, paragraph 3), becomes a strict junior homonym and is invalid. As well as afflicting the young lad with a used name, it is also incorrectly formed according to the rules governing construction of names. Being the first scientist (of truly international standing) to have spotted this error, I claim my right to propose a replacement name. I have chosen:

Tilburyanus inhirsutus arabicum Branch 1989
You will note that the Generic name is now correctly Latinized and the ending is more appropriate (being his most obvious feature for the moment!). The specific epithet also refers to the sub-adult plumage, while the sub-specific name is a traditional, uninspiring geographical allocation. Knowing that he is now correctly named, you may re-apply for birth certificates, passports and driving licences etc.”

For a man who played with snakes, Bill had a simple philosophy. Respect them and you won’t get bitten, and as far as I know, apart from a single dry bite from a *Thelotornis*, he never did. I remember the day that I brought a small shiny black snake all the way from the DRC [Democratic Republic of the Congo] to Bill’s home, and proudly handed him the blue cotton bag that contained the snake which I had carefully nurtured for the previous month or so. Bill gleefully but carefully opened the bag and peered inside. Then to my horror, he inserted his hand into the bag to retrieve the snake.

I said ‘Whoa! Hang on there a minute; I just want to get out my notebook and camera to record the first bite from this unknown species of *Atractaspis*.’ Bill pulled out his hand, the snake dangling limply between his fingers. Rigor mortis had already worn off.

“You’ve killed it” I said.

“No I didn’t.....Do you think it is a Norwegian Blue?” (A joke that can only be appreciated by followers of Monty Python).

But it was not only herps that Bill would talk about. As much as he was a scientist, he was also a profoundly loved family man who would talk with pride as much about his loved ones as he would about his work. Give him half a chance and he would talk for hours about his

sons Robbie, Matthew, and Tom and his new family—Nicole, Anthony, and James.

It was in 2012 that Bill first realised that he was only human after all, when he contracted malaria in Mozambique. That nearly finished him off. He eventually bounced back to his old self, but he was unable to dodge the bullet of MND [motor neuron disease]. When Bill contacted me in February 2018 to say that he had been diagnosed, it felt as if a tree had fallen on me. I had the good fortune to be able to spend several days with Bill over the last months of his life; and to be able to share memories of the good times, laugh together, discuss the iniquities of life, and to acknowledge the simple fact that we are all just fulfilling our biological destiny—albeit in different ways.

One cannot write about Bill without acknowledging the major part in his life that Donvé played, as his partner and soul mate, and in turn appreciate the huge hole that has been left behind by his passing in Donvé's life. In one conversation we had, we both agreed that it was one of the greatest privileges of life to be able to love and be loved back unconditionally. I don't think that anyone can overestimate the enormity of this gift. She made him so happy and in the end, so sad that his Dove would have to endure the last days of his life with him in the state he was in.

Bill asked me to sign as witness to his living will to not be placed on any mechanical machine that would prolong his life. As his MND advanced, even in the late stages, in spite of his body being totally paralysed, his mind was as lively as ever; as he fought day by day to extract, utilise, and enjoy to the last moment every second that was left to him. He was immensely saddened and so disappointed that he had run out of time to complete all the many projects that he was part of or had initiated. His illness had quite literally pulled the rug out from beneath his feet. I know that Bill handed over many of these to colleagues to finish—we should make him proud. Even as he inexorably neared the end, he was so brave in facing his fate. He could still make jokes about this. He once compared himself as a likeness of the blue-headed agamid that was named after him (*Acanthocercus*

branchi). Finally in the afternoon of 14 October, dulled by the ever-increasing CO₂ levels, he finally and peacefully breathed his last. The end to a magnificent life. His was an act we could all learn something from.

More than anyone, Bill understood and appreciated the fact that no one gets to live forever, but that everyone is hopefully gifted with the opportunity to leave a footprint embedded in the rocks of humanity—a footprint that will endure with a permanent relevance to those who follow one's trail. Bill had big feet for such a small frame, and no doubt we will be following his prints for many a year. I can only say that I was privileged to know Bill, and even more so, to think that he might have considered me to be a friend.

The memories of Bill will be enduring and he will always be celebrated as one of the world's leading herpetologists of our time. He will be sorely missed and long remembered.

Alan Channing

University of the Western Cape/North-West University, South Africa

I met Bill at a herp meeting while he was working at the Atomic Energy Board in the 1970s. He was hugely enthusiastic and well-read. Later, I was happy to support his application for the post of Herpetologist at the Port Elizabeth Museum, when asked by the Director. We undertook many field trips together, and for a while we formed a collaboration for funding from the forerunner of the National Research Foundation.

Although Bill and I worked on different groups, there was always a lot of friendly banter between us. His sense of humour was displayed on one field trip to northern Namibia, when he offered to cook the potatoes, while I prepared the meat. When it came time to eat, the potatoes were still crunchy. Bill's response was to explain that that was how they were cooked in Cornwall, and that it was a classical culinary procedure!

I will miss Bill's insights and our regular email exchanges. He provided a number of excellent photos for the upcoming book *Frogs and other Amphibians of Africa*, and was always willing to help, or offer a beer and a meal, when I was in Port Elizabeth.



Fig. 1. Cover image of *Hyperolius raymondi* used for *Frogs and other Amphibians of Africa* (Photo: Bill Branch).



Fig. 2. Bill photographing a lizard in southern Angola, 18 January 2009 (Photo: Alan Channing).

Dot Hall (Pitman, Basson)***Port Elizabeth Museum (Bayworld), South Africa***

A flood of memories flow through my mind when I reminisce on the very small part of Bill's life I shared. One in particular always makes me smile. When Bill joined the Museum in 1978, he was a complete unknown. We were observing his introduction to the staff with interest: A quiet, rather serious little man? From his first day he was a regular library user. He was passionate about books. Each visit he made to it was a learning experience for me. He freely shared his knowledge and always stretched my way of thinking.

On one memorable morning, shortly after he had joined the staff, all was quiet in the library when a strange scuffling noise caught my attention. No-one was in the library, so I put this observation down to my imagination and continued working. The same noise recurred several times till I eventually decided to investigate. There was a solid counter that separated the librarian from those using the library. I peered over this counter to find Bill on all fours, crawling behind a large leguaan [varanid] holding its tail and trying to direct it around the corner to my desk. I guess it was being a little uncooperative and his full attention was required for him to achieve his goal—"Frighten this librarian out of her mind!!!" After observing the scene for a short while I decided to launch a surprise "attack" from the back and gave him a pinch on his rear end. His reaction was marvellous. The leguaan was let loose and his fright was complete.



Fig. 3. Bill Xeroxing a puffadder to make counting of scales easier, to the disgrace of the librarian (Photo: Dot Pitman).

We both enjoyed sharing this amusing moment. How many million more smiles has he given to the vast number of people with whom he associated?

Rick Shine***Macquarie University, Australia***

I first met Bill Branch on the morning of Tuesday the 5th of September 1989, at the British Museum of Natural History (now the Natural History Museum London). Like me, he had travelled to the UK to attend the First World Congress of Herpetology, and like me, he took advantage of the opportunity to visit the British Museum of Natural History. Bill was looking for type specimens of African herps, and I was attempting to track down the reptile specimens that Charles Darwin collected in Australia during the voyage of the Beagle. As we sat and talked over lunch, I was astonished at Bill's breadth of knowledge about the African herpetofauna, and his intimate familiarity with the scientific literature on those animals. But I had no idea that we would end up as collaborators on a major project.

Five years later, I took my first (and only!) sabbatical from the University of Sydney. My wife Terri and I had always wanted to see the famous game reserves of southern Africa, and our oldest son was about to turn 12—after which time he would have to pay a full fare on the airlines rather than half-price! So I contacted Bill about the possibility of dissecting preserved snakes in African museum collections for ecological data



Fig. 4. A miserable but memorable visit with Bill to Bird Island to look for reptiles, we never found any (Photo: Dot Pitman).

(gut contents, gonads, etc.) as I had done for snakes in Australian museums over the previous years. Bill was enthusiastic, but thought that the best collection might be in Namibia, where over 1,500 snakes that had drowned in an open canal (the Eastern Water Carrier) had been preserved by the local wildlife authorities. And with the first South African elections looming, and political unrest likely as power shifted from ‘*Afrikaanders*’ to native Africans, Namibia looked like a quieter, safer option than South Africa for a family with 10-year-old and 2-year-old children.

We flew to Namibia while Bill drove the museum’s Kombi-van from Port Elizabeth to Windhoek to meet us. And being Bill, he had a much better idea than staying in Windhoek to dissect the snakes—instead, we piled them into the van and took off for Gobabeb, where we could enjoy the spectacular dunes in between long hours of peering inside dead snakes. The Aussie team (me, Peter Harlow, and Jonno Webb) peered inside the innards of dead snakes and called out numbers, while Terri wrote them all down into data-sheets. Bill carefully examined every half-digested frog and reptile that came out of a snake stomach, almost always managing to ID it, even if he only had a few toes to work with. It was a happy and effective team.

After we finished the Namibian snakes, my family flew off to the USA while the rest of us drove down to Pretoria to look at MORE snakes at the national museum in Pretoria. It was a classic herp “road trip,” with frequent detours to look for specific taxa (usually, so that Bill could get a photograph for his field guide). We made an obligatory stop at Poffadder (= “Puff Adder”) one of the few towns named after a snake, near the border between South Africa and Namibia. A photograph I took on the town’s outskirts captures the relaxed joy of herpetological zealots indulging their passions (Fig. 5). We worked long hours in Pretoria, obtaining a mountain of data that eventually translated into 15 papers on the natural history of several major lineages of African snakes. We also sampled the local beer and watched World Cup soccer games at bars downtown—horrifying some of the locals who were convinced that we would be mugged as we walked the streets at night.

Throughout this first African adventure, Bill was fantastic. Extraordinarily knowledgeable, with a vast network of contacts, he made the project possible. We talked long and often about everything from fishing to the mysteries of bureaucracies and families—and especially, about snakes. Hopping off a plane and looking inside preserved specimens can generate a lot of data—but it was Bill’s long experience that enabled us to put that information into context. For many of the species about which we wrote papers, I had never even seen a live specimen—but Bill had, and his firsthand knowledge helped him to laugh off my ill-informed speculations, and keep our interpretations true to the reality of snake ecology in southern Africa. Bill was a terrific collaborator



Fig. 5. Jonno Webb, Bill Branch, and Peter Harlow posing at the outskirts of the town of Poffadder in northern South Africa, reveling in the idea that somebody actually named a town after a snake (Photo: Rick Shine).

and a wonderful friend. I feel privileged to have been able to work with him.

James B. Murphy

Division of Amphibians & Reptiles, National Museum of Natural History, Washington DC, USA

When we first met at a herpetological conference in the US many years ago, Bill and I noticed that our love of amphibians and reptiles, overall biological interests, and personal histories were strikingly parallel. One major difference was that Bill had completed a Ph.D. in chemistry and I barely passed my chemistry courses. Fortunately, he changed trajectories and excelled in herpetology. As we shared our stories over some beers until the break of dawn, Bill and I quickly bonded. I invited him to come to Dallas, Texas, where I was herp curator at the Dallas Zoo with a spacious guest room available in my home. As we toured the Zoo’s herp collection, Bill was delighted when he saw the large breeding group of New Caledonian Geckos (*Rhacodactylus leachianus*, Fig. 6). There was a particularly large and impressive male that was surplus, so I gave it to Bill—his stunned reaction and gratitude were wonderful to watch as he carefully packed the saurian to hand-carry it back to Port Elizabeth [ED note: This gecko is still alive in the Port Elizabeth

Museum as of July 2019].

After the First World Congress of Herpetology in Canterbury, UK, he invited me to stay at his parents' home nearby until we later went to Bonn, Germany, for the first Varanid Symposium held at the Museum Alexander Koenig (Fig. 7). In my view, Bill was a pretty stocky fellow, but his mother was concerned that he was not paying enough attention to proper nutrition, so she followed him for several days with handfuls of vegetables, insisting all the while that he was becoming a mere slip of a man. The scenario reminded me of a Monty Python skit.

At the varanid meeting, Bill presented a wonderful lecture on the White-throated Monitor (*Varanus albigularis*)—"The Regenia registers of Brown (1869–1909). Memoranda on a species of Monitor or Varan." Branch covered all aspects of Alfred 'Gogga' Brown's extensive observations—sex ratio, size, body proportions, hemipenial morphology, visceral fat bodies, coloration, diet, cause of death, longevity, reproduction, gestation period, egg laying, oviposition, eggs, clutch size, hatchling size, incubation period, growth, behavior, mating behavior, shedding, thermoregulation, predation, parasites, exploitation, and seasonal activity and retreats. The amount of information that Gogga had collected on his captive lizards and in wild counterparts in the late 19th century is truly astounding.

Over time, Bill sent a number of African and Namibian reptiles for the Dallas Zoo collection, including Angulate Tortoise (*Chersina angulata*), Parrot-beaked Tortoise (*Homopus areolatus*), Tent Tortoise (*Psammobates tentorius*), Mountain Adder (*Bitis atropos*), Dwarf Adder (*Bitis rubida*), Many-horned Adder (*Bitis cornuta*), Cape Dwarf Chameleon (*Bradypodion pumilum*), Lesser Flat Lizard (*Platysaurus guttatus*), and Drakensberg Crag Lizard (*Pseudocordylus subviridis*).

In the ensuing years, we spent much time together at meetings and he shared his concern about shrinking funding for the Port Elizabeth Snake Park and Museum. Frederick William FitzSimons (born 1875) was the first Director of the Museum in 1906, and he developed the



Fig. 6. Male New Caledonian Geckos (*Rhacodactylus leachianus*) still alive in Port Elizabeth Snake Park (Photo: Werner Conradie).



Fig. 7. Varanid Symposium participants at Museum Alexander Koenig in 1991. Bill Branch is the seventh person from the left, in the front row.

Snake Park. His son, Vivian, assisted him and both of them published in herpetology. His younger brother, Desmond C. FitzSimons, started the Durban Snake Park. F. W. FitzSimons also wrote books on the natural history of South African mammals, including primates.

Bill was a consummate biologist whose contributions to our knowledge of African amphibians and reptiles over several decades set the high standard for herpetological work. His nominal retirement as curator of herpetology at the Port Elizabeth Museum occurred after many years of service. As far as I know, he did not free-handle venomous snakes nor put them on his head. Every time we met, I could be confident that he would cover subjects virtually unknown to me. He will be missed.

Luke Verburgt

Enviro-Insight & University of Pretoria, South Africa

Bill replied to my email almost instantly and in great detail! I'd been very hesitant to contact my herpetological idol about a reptile identification query, because I guess I was afraid to disturb such an important person with possibly silly and trivial queries from me, a nobody. Yet to my delight, Bill took the time to carefully answer my questions, providing great detail and assistance. No admonishment for not having read the appropriate books/papers, and no arrogant stance regarding my lack of herping credentials! I was thrilled, and it opened up communication between us to such an extent that soon we were communicating about African herpetofauna via email quite regularly, with Bill always helpful and kind in dispensing his amazing wealth of knowledge. Like a mentor really.

I eventually met Bill in person months later in Namibia, along with Johan Marais and Aaron Bauer, while they were on a collecting field trip. It was such an honour to be sitting around the same table as these herping heroes, and I was rather star-struck. After some fieldwork with the team, I picked up on the fact that the species of *Rhoptropus* that we were collecting was not the one I had expected to be there according to Bill's field guide, which was really my main source of herp

knowledge, as it has also been for so many others. I eventually plucked up the courage and cautiously approached Bill one afternoon, to ask about this *Rhoptropus* situation. He laughed heartily and said, “Oh, that map is complete rubbish!” I couldn't believe it. The author of the book that I cherished above all others just told me that some of it wasn't the complete truth! And right there I learned two massive lessons from Bill about African herpetology: imperfect data from under-sampling abound, and not being afraid to question the existing understanding.

Sadly, that was the one and only time I got to be in the field with Bill, and it was far too brief. Thankfully though, we collaborated a lot after that with several resulting papers where I got to be a co-author with Bill—a huge honour! However, the greatest honour for me in this regard was having Bill as a co-author guiding my very first reptile species description, an interaction through which I learnt more than I could've ever imagined. He took what was a pretty ordinary and mundane manuscript and guided me on how to improve it to an acceptable standard, the standard which he was instrumental in setting for African herpetology.

After that I regularly reported to Bill, who was always extremely interested in my findings because I was often working in poorly sampled rural places across Africa. In his now familiar mentor role, he would encourage me to do as much useful sampling as possible, and also to think harder about why a particular species was observed in the habitat I found it in and, therefore, to consider its ecology in greater detail and gain more insight from my observations. In short, Bill made me a better herpetologist and I am forever grateful for his friendship and his mentoring.

Although I didn't see Bill in person very often, it was always a treat to hang out with him and his fantastic sense of humour. But what I think I enjoyed the most was to hang out with him and to see him having such fun at the 2017 HAA conference at Bonamanzi, and I was even lucky enough to win a “selfie” with him! Unfortunately, I never actually received the “selfie”—but fortunately, Shivan Parusnath managed to capture the “selfie”-taking moment perfectly, and it is my favourite photo of Bill and myself (Fig. 8).

I received the news that Bill had passed away while I was sampling in the Cabinda Province of Angola, an area of great interest for Bill. While we all had known for some time that it was an inevitability, the news of his passing came as a massive shock to me because only a few hours earlier, during his last night, Bill had somehow managed to send me a lengthy Whatsapp message, instructing me to collect as many DNA samples of certain species as I could due to the importance of the sampling locality I was in. And thinking about it now, that's just how it was always going to be for Bill, the ever-enthusiastic herpetologist and helpful mentor to the very, very end. Rest easy Bill, I miss you so much



Fig. 8. Taking a “selfie” with Bill as part of the prize for runner-up best photographer (Photo: Shivan Parusnath).

and hope that I am able to justify the effort you put into sharing your time and knowledge with me.

Julian Bayliss

Ecologist and Explorer, Wales

I first met Bill when he came to undertake a herpetological survey on Mount Mulanje in Malawi with Johan Marais and Michael Cunningham in 2005, as part of the ongoing ecological monitoring programme on Mulanje that I was coordinating. However, it was really when we met the second time, when Bill and Werner Conradie joined me on Mount Mabu in 2009, that we really got to know each other well. I had been working the mountains of northern Mozambique for several years prior to this event, and had managed to turn up several new species of snakes and chameleons, although my herp work was opportunistic (I discovered *Atheris mabuensis* by stepping on it!) and I needed professional assistance (Fig. 9). These discoveries attracted Bill, and we arranged for a trip to Mt. Mabu forest to collect more specimens, and also to see if we could collect specimens of a *Nadzikambia* chameleon that was only known from a couple of photographs taken on my previous visits. We were successful in this endeavour, and I managed to collect the first specimen of the *Nadzikambia* from Mt. Mabu which Bill named after me as *Nadzikambia*



Fig. 9. Photograph of *Atheris mabuensis* taken by Bill—probably the best photograph of a snake I have ever seen (Photo: Bill Branch).



Fig. 10. The Mt. Mabu 2009 science team. Left to right: Werner Conradie, Martin Hassan, Julian Bayliss, Bill Branch, Hassam Patel, Colin Congdon, and Steve Collins (Photo: Julian Bayliss).

baylissi. I was deeply honoured by this gesture.

The 2009 Mt. Mabu expedition proved to be a very enjoyable expedition, packed full of laughter, good company, and good food. I had also invited the butterfly crowd from the African Butterfly Research Institute (ABRI), a great bunch of eccentrics, and the stories flowed around the camp fires at night. At the end of the expedition, we all stood below a large tree on the forest camp in Mt. Mabu with Bill at centre stage (Fig. 10). This is one of my favourite photos of Bill, and it captures a moment in time where nothing outside that camp at that time really mattered. This was the start of a very good friendship with Bill (and Werner) and some great correspondents. However, one of my fondest memories of Bill was spending time with him in the Mt. Nimba forest in Liberia. It was part of an EIA on a proposed mining concession, and it was just the two of us for several days, which gave us plenty of time for chewing the fat; especially when we talked about rugby and Wales vs. South Africa or England, as I am from Wales and Bill was originally from England, and then South Africa. At that time, I had flown up from a festival in South Africa and brought with me a ‘Green Policemen’ helmet which Bill dually wore (Fig. 10, this photograph shows Bill beaming a big smile).

Bill, I will miss you greatly—you were an inspiration to me. Not only did you teach me a lot about reptiles, but you were also a professional in everything else you did. An expert and a gentleman. In the last communication I received from Bill, a couple of months before he passed, he told me ‘not to defer my dreams’—advice which is applicable to us all and advice I intend to follow.

Michael F. Bates

Department of Herpetology, National Museum, Bloemfontein, South Africa

I knew about Bill soon after I started working at the National Museum in Bloemfontein in 1983, as he was then editor of the Herpetological Association of Africa’s journal. The first time I met him was at the HAA’s first



Fig. 11. Bill Branch in the Nimba forests close to Nimba Mountain, Liberia (November 2011). Bill is wearing the green policeman hat I had brought with me from South Africa (Photo: Julian Bayliss).

conference held at Stellenbosch University in 1987. I was only 25 at the time, and Bill was about 41, still quite slim and with a full head of black hair! At that time he was busy wrapping up work on the first edition of his famous reptile field guide. Even then I remember Bill having a certain charm about him and the aura of a man with a deep knowledge of his subject matter.

Over the years I visited Port Elizabeth Museum several times to examine specimens for various research projects, including some on which I collaborated with Bill. Having him all to myself and available to answer my barrage of questions was always special. However, I think my fondest memories were in the early 2010s when we spent considerable amounts of time editing the text for the *Atlas and Red List of the Reptiles of South Africa, Lesotho and Swaziland* (published in 2014). As first and second (Bill) editors, the bulk of the editing fell on us. I would, for example, e-mail Bill the text for a species account and ask such questions as “is it still regarded as a subspecies” or “has anything been published about this recently.” I could count on him to respond within a day or two, and his responses were always insightful. He seemed always to be up-to-date with the latest taxonomy and the most recent literature. And so it was that we e-mailed the various sections of text back-and-forth until we were both happy. I have very good memories of those times.

Another special memory I have of Bill was in May 2018, a few months after he was diagnosed with MND, when I visited him at home in Port Elizabeth, together with Aaron Bauer and Marius Burger. By this time he was, for the most part, wheelchair-bound. Nevertheless, he was as talkative and interesting as ever, especially with regard to herpetological matters, and he also exhibited his usual great sense of humour. We spent most of the time at the computer in his study where he showed us photographs of interesting and new reptiles, and of field trips he had conducted with various colleagues over the years. Also, I brought him a copy of a recent taxonomic paper on egg-eating snakes (Bates & Broadley) that



Fig. 12. Michael Bates (left) with Bill Branch and Darren Pietersen during the Herpetological Association of Africa's conference in Pretoria in 2013 (Photo: W.R. Schmidt).

had just been published in the National Museum's journal *Indago*. The front cover of the journal featured a montage of Bill's excellent colour photographs of these snakes, and it gave me great pleasure to see how pleased he was with the way it turned out.

Bill had an enormous presence in the field of African herpetology. He impressed me as a very well-read man, and this was reflected in his wide and seemingly limitless knowledge of reptiles and amphibians. Bill was always willing to share PDFs of research articles and in this way he helped me on innumerable occasions. Also, I was inspired by his style of writing and attention to detail. I still think about Bill often and will miss him for several reasons, not least for the fact that his expertise was always just an e-mail away.

Pedro Vaz Pinto

Kissama Foundation, Luanda, Angola & CIBIO-InBIO, University of Porto, Portugal

I first met Bill in January 2009 in the most appropriate of places: deep in the Angolan Namib desert, in Iona National Park. We were part of a large group of scientists assembled by Brian Huntley for a biodiversity expedition in southern Angola. I remember approaching Bill after dinner in the camp site, and he was keen to see my photo files and became interested in some bush viper pictures, which led to a few engaging stories and discussions. At that point I was simply curious about reptiles, and more involved with furry or feathered creatures. The following day, I drove my Land Cruiser to where I could see Bill and his colleagues had parked their pick-up truck next to some granite boulders. I could sense some excitement in the party, so I asked Bill what they were doing. He invited me to join them and opened a little box to retrieve a tiny beautiful little gecko, one of the gems of Angolan herpetology which was not even formally described at the time: the endemic Plume-tailed Gecko, *Kolekamus plumicaudus*! He then showed what was special and unique about that species and chatted about other leaf-toed geckos. I was fascinated of course, and it was quite an introduction to reptiles. Over



Fig. 13. Bill Branch processing specimens in the fading light of the Angolan Koakoveld (Photo: Pedro Vaz Pinto).

the following years we would become good friends, but looking back I'm still amazed to realize how generous he was by sharing that amazing find with someone he had just met the previous day. Other scientists would have kept their cards very close to the chest. But Bill kindly drew me towards the world of herpetology for which I'm forever indebted, but above all I believe he made me a better scientist and better naturalist. He taught me to make an effort at looking into the bigger picture, to see the multiple layers and connections that lie hidden behind the outer surface of a given ecological theme.

My best memories with Bill, without any shadow of doubt, were the days in which I was privileged enough to travel with him to some of the most remote corners, wildest places, and biodiversity hotspots in Angola. We would typically look for a scenic landscape off the beaten track and choose our camping spot. Some of the time shared with Bill, around the campfire in the Angolan desert, mountains, or forests, was memorable. Our camping expeditions were hugely stimulating scientifically, exciting and unpredictable, and very importantly, always bathed by loads of good humor! These expeditions could be physically exhausting, but soon after I was looking forward for the next trip with Bill.

Other scientists are much better prepared to praise Bill's unique and extraordinary legacy to African herpetology. I can add that he did leave a crucial mark on Angolan herpetology, but tragically with his premature passing away, it wasn't allowed to further crystalize during his life. He was arguably the most influential herpetologist to have worked in Angola for a sustained period, and is the main person responsible for bringing herpetology into the biodiversity agenda in modern Angola. I have no doubt that his pioneering role will be recognized in the future by young Angolan biologists. On a personal note, whenever we came across a new lizard or snake, I got used to my sons asking me 'Will Bill want this specimen?', 'Has Bill identified this species?', 'Does Bill need more specimens?' and as result, these now rhetorical questions remain quite vivid



Fig. 14. Bill Branch photographing a Jameson Mamba in Angola with Ninda Baptista (Photo: Pedro Vaz Pinto).

and still drive me on my searches. There are still a lot of ‘goodies’ that we will catch for you Bill, and that’s a promise!

Kirsty Kyle

KwaZulu-Natal, South Africa

I had the good fortune of growing up at Kosi Bay with Bill Branch as a much loved family friend. Bill had gotten to know my parents, who were the resident scientist and his wife for the area. In those days, he did an almost annual foray to Zululand, what with it being such an interesting part of the country for herpetofauna. Whenever he moved through the area, with his pack of scientists, they would use our house as a base, and for my two older brothers and I this was just the best thing ever. His trips became the highlight of our year and I think he thoroughly enjoyed having three young, able-bodied slaves, ever so willing to dive after any reptile that was silly enough to stick its nose out in our vicinity. A friendly disagreement developed as we got older and started objecting to his pickling tendencies. In the later trips we would “not see” a lot of the more common species because dear old Uncle Bill would just pickle anything we presented him with, which was a bit hard on our budding conservationist hearts. Although we had a pretty much genetic interest in herps, I think those times with Bill were extremely formative in all three of our lives, they certainly were in mine. The fact that he was interested and enthusiastic in teaching and encouraging a little blonde thug of three years old in the ways of reptiles was amazing.

Bill was absolutely instrumental in setting me on the path I am on today. Throughout childhood it was a privilege to spend time in the field with him and just absorb all the information he so generously and freely dished out. It had a major impact on my interest in herps. I emailed back and forth with him whenever I found something interesting, and I sent him pictures of all sorts of different reptiles over the years and he would always respond in his warm, friendly, and encouraging manner, which was just amazing. My favourite memory of Bill would have to be on his last visit a few years back,

when he proudly presented us with a beautiful pot that Donvé had made, decorated with an aloe he informed us he’d just plucked from our outdoor lizard enclosure. There were no flies on Bill and I loved that about him, he always told you the truth, even if it put him in not the best of lights. We still have the aloe in the pot.

I wish I had a picture with him from the early days because it really would be a cute one. I fondly remember parking on his lap as a very little girl, discussing whatever, feeling terribly important, with his black mop of curls and my blonde mop of curls. It would have been such a cute picture. I miss Uncle Bill, the world in general is a lot less fun without him and my Facebook is a much darker place without his frequent updates, pictures, thought processes, and quips. I hope he forgives me for specialising in amphibians instead of reptiles, and I’m incredibly grateful to have had him as a friend, as well as a mentor.

Krystal Tolley

South African National Biodiversity Institute, South Africa

I knew about Bill before I moved to South Africa in 2001, as he and Colin Tilbury had some chameleon DNA samples for my upcoming postdoc project. The project almost didn’t happen, as Bill and Colin got cold feet, but when I arrived I learned that they decided to let me give it a try. Their trust in a stranger with whom they had never worked ended up building a friendship and collaboration that lasted nearly two decades. As that project progressed and more projects arose, Bill encouraged and supported me both in a personal and a professional capacity. In fact, the entire herpetological community welcomed me, something that I was not used to, coming from the competitive world of marine biology in the northern hemisphere. Because of Bill, Colin and all the SA herpers, I felt like I had found a home that I didn’t want to leave, and Bill was instrumental in that. I cannot remember actually meeting Bill for the first time. My first distinct memories of hanging out with Bill and all the herpers is from the Port Elizabeth HAA conference in 2004. What sticks out in my mind is that at the concluding banquet, Bill received the Exceptional Contribution to African Herpetology Award and he was so touched by this that he wept. That spoke to his nature as a caring person who knew that strength and courage, not weakness, comes from personal relationships and bonds. And his connections with his friends are something he fostered.

I have many distinct and fond memories of Bill but strangely enough, most of them relate to our friendship, not to herpetology. When we would meet, the first things he would ask me about was how I was, how was my personal life, what was happening, was I happy? He had many wise words for me along those lines, giving advice, encouragement, and reassurances that eventually I would find my path. Then of course, the

‘herp talking’ would start. He would go on for hours, non-stop, about snakes mainly. Most times, the topics were just beyond me. I tried to absorb what he said, but there was so much information that my brain couldn’t handle it. I do remember that a long discussion about *Leptotyphlops* made me realise what cool things they are, and I still hope one day to actually work on them.

I was fortunate to have the chance to visit Bill shortly before he passed away. We both knew, as did his wife Donvé, that I was there to say a final goodbye. This was indeed the last time I saw him and it was emotional for everyone, but my memory is still a good one. The same old routine was there. He asked me about my life first, and he gave me wise words and insight about life. Then he spoke about herps (including *Leptotyphlops*) for about four hours non-stop. The thing that was different this time, was that he often interjected the conversation with things about himself. Dreams and wishes, failures, successes, lost opportunities. He talked a lot about how it’s important not to waste time on petty or destructive things in life. But to focus time and energy on the people in your life that care about you and to never take that for granted. He spoke about the balance between the work related passions of a herpetologist, and that this has to balance with life, friends, and family. Bill was a hard worker, but he did focus on family and friends, and I don’t think he took any of that for granted. The way that his first questions always related to our personal connection and friendship, and about which analysis I was running, speaks to that. The wisest words that Bill ever said to me are: “Friendship is a gift. It’s a gift that others chose to give, and that you chose to accept in whatever form it takes.” Bill gave that gift to me and to so many others. That is what I will remember him for the most.



Fig. 15. Bill Branch and Krystal Tolley in south-western Angola in 2009 (Photo: Krystal Tolley).

Mzi Mahola **South Africa**

I first met Bill when he arrived at the Museum. A year or two later, I invited him to join our Port Elizabeth Museum soccer team, which was playing in the Industrial League. He didn’t play many matches, because of his

commitments, but he was a good soccer player. A year later, I was transferred from my department to join and work with him as his research assistant. We often went to Sardinia Downs to tag, study, and monitor the movements, growth, and development of tortoises. After that he introduced me into his other research and study programme of other animals, such as frogs, snakes, and lizards.

One day we were going up the Zuurberg Mountains when he made a deal for us; “If you happen to catch a snake first, I will buy you a bottle of beer at the Zuurberg Inn on the way back; but if I make the first catch, you will buy me a bottle.” That was fair enough for me. Bill was at the wheel of our Land Rover. We were driving towards the forest at the foot of the mountain when I saw a female boomslang flying towards the forest. Bill noticed my hasty intention to open the door of the moving vehicle and he quietly said, “Forget it! Boomslangs are very shy; you’ll never catch it unless it is in a tree.” A few minutes afterwards, we left the Land Rover and with our hunting gear and went our separate ways. It took me less than five minutes before I heard the hissing sound of a slithering snake. I saw the disappearing tail of a rinkals entering a hole amongst rocks on a ledge. I put on my safety glasses and peeped into the hole, and saw the two shiny eyes watching the entrance. With my tools I pulled the snake out and put it in my canvas bag, and declared my victory to Bill. He didn’t believe me. It didn’t matter how many snakes he collected afterwards. I had beaten my master in his game and the bottle of beer would be a cherry on top.

We were on a trip to the Drakensburg Mountains and our first night stopover was in Centane, at my in-laws. We shared the same bedroom. At night, Bill said something, which I could not let pass unchallenged; “Kentani is the only place, in the Eastern Cape, that has no tortoises.”

“Why?” I asked, thinking that this had to do with the climatic environment.

“Africans ate all of them and left nothing to sustain these animals.”

“No! That is not true!” I protested, because I had a relationship with these people.

“What do you mean, it is not true? Dr. . . . (*I don’t remember his name*) learnt about this when he was investigating the cause of their depletion in this area, years back in the early twenties. I read his book and you can’t dispute it.”

“Well, his assumption was wrong.” I replied, confidently, knowing that what he was going to hear would shock him. “First of all; it is very, very difficult for a stranger to get information from traditional amaXhosa, because these people are known for their scepticism of strangers. If you ask them anything, they will ask, ‘why do you want to know?’ After that they will not share with you their knowledge; more so if you’re a stranger. In the past amaXhosa trained their children from an early age never to tell a stranger the truth, especially to white

people.”

Bill listened quietly, without interrupting.

I continued, “Now let me tell you something that they did not tell Dr.....? AmaXhosa did not eat any creeping or crawling animals, like centipedes, lizards, snakes, crabs, frogs, locusts, ants or tortoises, hence they looked down upon Khoisan people, because of their “repulsive” diet. The Khoisans ate these animals.

Even though they were converted into Christianity, there are still some Xhosa households who hunt and kill or keep tortoises for their strong religious or cultural beliefs. They generally believe that if they burn a tortoise shell in a kraal with cattle, the cattle will multiply. Cattle are a status symbol or a bank to our people. Tortoise shells are also used as troughs to store drinking water for chickens so that they may increase. There is also a belief that if live tortoises are kept in a household, they will repel evil spirits. These beliefs surely must have been the cause of depletion of these animals in an area as conservative and traditional as Centane. I was told that because of their scarcity, locals are prepared to purchase and import them from other areas.”

“It makes sense,” Bill said and kept quiet for a long time afterwards.

Working with Bill had a very strong impact on me. He was very dedicated and committed in whatever he was doing. In Matatiele, he went out into the night to search for frogs in the river while it was raining and thundering. He didn’t allow anything to stand in his way. Many years later, after I had left P. E. [Port Elizabeth] Museum, I went on a personal and voluntary excursion of documenting and taking pictures of bushmen paintings in the caves in the Nkonkobe and Chris Hani Municipalities. Without his basic research training I wouldn’t have embarked on this project. Bill gave me a hands-on experience in researching and I thank him for sharing his skill with me.



Fig. 16. Bill Branch discussing the finer points of the day’s photographic record of collections with colleagues and students, Lagoa Carumbo, May 2012. (Photo: Brian Huntley).

Brian J. Huntley

South Africa

During 2009 and 2012 I had the pleasure of introducing Bill Branch and a few dozen other field biologists to the diversity of life in the deserts, montane grasslands, miombo woodlands, forests, and floodplains of the far reaches of Angola. Bill soon proved to be the hardest working and most convivial member of the teams, which comprised up to 30 biologists from ten countries.

During the first expedition to southwestern Angola, in January 2009, we camped out on the Humpata highlands and in the Namib desert. Here Bill and fellow herpetologists found multiple new records and several new species of frogs and lizards. From the faint light of dawn to the pitch darkness of night Bill would be in the field or at the makeshift laboratory tent, where a generous donation of Cuca beer from the local brewery kept spirits and laughter levels high. What impressed me most about Bill was his ability to inspire all around him— young students to ageing professors—game rangers to army generals—with fascinating stories about his cold-blooded friends.

In May 2012, when we were camped out along a Congo tributary at Lagoa Carumbo, in the far northeast of Angola, the evening’s discussions around the campfire ranged from Bill’s erudite interpretation of current species concepts to scary personal experiences of snake bites and the treatment thereof. We were eight hours drive from the closest town, and another five hours from the closest doctor. One afternoon, Bill had been out to set a trap for a black mamba that had been seen slithering down a hole on a rock face. He casually told us how he had, that same morning, pulled what he thought was a harmless water snake out of the Luele River. Only when he returned to camp did he discover that it was a new elapid record for Angola – Banded Water Cobra, *Naja annulata*.

We had planned to visit Portugal together early in 2018 to discuss collaborative projects with colleagues at the University of Porto, but at a meeting in Cape Town that January, Bill informed me of the advice his doctor had given him that week: he should not travel. We soon learnt of the severity of his illness, but this did not slow Bill down. He was already under heavy pressure to complete his catalogue of *Snakes of Angola*, but did not hesitate to honour his promise of a chapter on reptiles for the synthesis volume that I was coordinating on *Biodiversity of Angola*. We kept up a lively correspondence to the end, his sharp wit never failing. Fittingly, given Bill’s tremendous role in inspiring young researchers in Angola, the synthesis volume includes a dedication to him.

Roger Bills

South African Institute for Aquatic Biodiversity, South Africa

I first met Bill in Marromeu, central Mozambique. We were part of a team lead by Jonathan Timberlake looking at the biota of the lower Zambezi’s delta region. I had

driven up from Grahamstown with a bakkie and trailer, and had slept in the car over several nights due to poor road conditions and slow progress. Basically, I was exhausted. This did not get me any respite from Bill's sharp humour and I had to quickly shape up.

For me the trip was tremendous—there was water everywhere despite it being the dry season, and an abundance of fishes and the fauna was mostly new to me. For most of the other zoologists it was not the best season and consequently a bit frustrating. Bill and I got on from the start and we spent time out in the dry fields with a large bulldozer that was flattening termite mounds looking for snakes and lungfishes and several days on a boat going down the Zambezi.

The boat trip down the Zambezi was supposed to be an overnight affair—down the Zambezi to the mouth, a channel through mangroves to one of the delta's southern braids and up to the small village of Malingapanzi. Unfortunately we missed the tide and left late, and went down river on an incoming tide. It took us the whole day to get down to the mouth where we camped at a fishing village overnight. We expected to get going at first light but the local fishermen stole our rudder as they wanted payment for camping. It took our Mozambique counterparts the whole morning of negotiating and refitting the rudder before we could leave. The time however was well spent: Bill went fishing (he was a good angler) and caught our only *Glossogobius giuris* for the trip, and I caught a load of mud-skippers in the mangrove flats. Our delay meant we missed the tide again and going up the southern channel to Malingapanzi was against the outgoing tide. We got there late on the second day—Bill had caught one puff adder. He wasn't very happy and did not return by boat the following day.

From all my experiences with Bill, the impressive thing about him was his resourcefulness in the field, whether collecting by himself or soliciting samples from locals, he managed to get incredible numbers of samples. Returning to camps in the evenings would invariably find Bill at a table covered with specimens that he would be fixing, photographing and taking tissue samples from. He

spent long hours doing this work. On one trip to a sand mining project near Pebane, Mozambique, we fell afoul of this. Bill had been there the week before and the locals were used to giving reptiles they had caught to passing vehicles. On our drive from the airstrip to the exploration camp we were oblivious of this. After the second snake came through the window in a flimsy plastic bag, we wound up our windows and did not stop anymore!

Bill was an incredible intellect, a world-class scientist but far more importantly a great guy. It was a privilege to have spent time with him, my life is richer for it.

Johan Marais

African Snakebite Institute, South Africa

Back in 1980, while I was curator of Transvaal Snake Park, I met Bill during one of his visits but we barely spoke. I was a youngster cleaning snake cages and Bill was visiting Rod Patterson and Anthony Bannister. We often corresponded and I supplied Bill with a bunch of photographs for his field guide, but it was only in early 2000, on a field trip to Namibia, that we really bonded.

We did several field trips to Namibia, often with Aaron Bauer, but our trips to Niassa in northern Mozambique, Mulanje Mountain in Malawi, and southern Angola were memorable. Field trips are special as there is ample time to chat, especially when driving long distances. I particularly enjoyed the chats with both Aaron and Bill, and although there were endless topics discussed it was largely about reptiles. I often wound Bill up about photographing reptiles on inappropriate props like fruit and flowers that were out of place, and he accused me of taking rather poor photographs as I had a bad eye.

His wry sense of humour brightened things up on those long journeys and he was particularly good at irritating Aaron, not to mention times when he would lose specimens while photographing them! My best Bill moment: when an American missionary's wife in Nampula asked Bill what he does for a living, he responded that he was a reptile scientist who did field work, described recently discovered reptiles, and wrote scientific papers about his discoveries. She responded:



Fig. 17. Bill and Anton Bok at the Kalumbila Mine Camp, Mwinilunga District, North-West Province, Zambia, May 2010 (Photo: Roger Bills).



Fig. 18. Johan Marais and Bill Branch with a Rock Monitor in Namibia. (Photo: Jackie Childers).

‘Yes but what is your real job?’

It is hard to grasp the gap that Bill has left behind, and so many of us miss the times that we could call or drop him an E-mail. He was notoriously bad at responding to E-mails so I got into the habit of numbering my questions. Needless to say, Bill would only answer those he felt like answering.

Mark-Oliver Rödel

Museum für Naturkunde, Germany

My first contact with Bill was in 1996. He asked for a copy of my frog book, and invited me to give a talk on West African amphibians and reptiles on the third World Congress of Herpetology in Prague, where I met him for the first time in person. Bill was organizing a session to summarize the progress in African herpetology. Thus, it was Bill ‘officially’ introducing me, my Ph.D. not yet finished, to the community of African herpetologists. We kept contact thereafter, but it took a few years until we met again.

Following a workshop to define conservation priorities for West Africa, Conservation International started a series of rapid biodiversity assessments in little known areas across the Upper Guinea forests. In early 2002, Bill and I were asked to participate on one of these RAPs, targeting the Haute Dodo and Cavally Forest reserves in western Côte d’Ivoire. He was responsible for the reptiles and I was to focus on amphibians, but of course we conducted all field work together, recorded many interesting amphibian and reptile species, ignored all CI safety rules, and had a lot of fun catching animals and talking rubbish. For Bill it was his first time being in West Africa, and his first time working in rainforests (as a ‘typical’ South African he showed up in shorts and it took me quite a bit to convince him that working in a rainforest in shorts is a very stupid idea).

Not all of the experiences were fun. In one night in the Cavally forest, we walked far from camp and encountered a few rarer species we hadn’t seen before on the trip. On our way back, we stumbled straight into the largest raid of army ants (*Dorylus* sp.) I ever encountered! The forest floor and all lower parts of the shrubs and trees were covered with these aggressive insects, and in seconds the ants were everywhere on and under our clothes. We just ran to leave them behind, and then had to strip naked to pull off hundreds of ants, all holding onto the skin they had successfully penetrated with their sharp mandibles. It was only when we finally finished them all off (one has to pinch off the heads of every single one) and turned again towards the campsite, that Bill realized that he had lost his glasses. We had to turn back into the ants to search for them..... A much more pleasant experience on that trip was when we found the first live caecilian, *Geotrypetes seraphini*, Bill had ever seen.

Caecilians were also one of the most spectacular findings, actually the first country record for the entire group, the next time we met. In the fall of 2003, Bill

invited Johan Marais and I to survey amphibians and reptiles in the Niassa Game Reserve, in northern Mozambique. Although it was the core dry season it was an extremely successful survey, revealing 57 reptile and 31 amphibian species, including a new *Cordylus*, and further potentially undescribed species including the *Scolecophorus* mentioned above.

Thereafter we met regularly, mostly in South Africa, but a few times in Germany as well. Bill often took me on shorter excursions across southern Africa, e.g., showing me spectacular parts of the Cape Fold Mountains or the Karoo, and I frequently visited him and his wife Donvė in their amazing house and garden in Port Elisabeth. There we had long and entertaining discussions about herpetology, science, politics, or sports, while sipping on a nice glass of wine, observing the many birds in the garden, or following a soccer or rugby match on television. We never agreed on which soccer team or player was worth supporting, and I could always bet that I would receive a derisive email after a German defeat against an English team in the Champions League. Bill was mad about some sports and missing an important rugby match was impossible, even on an excursion. Particularly memorable was when we once drove through the Karoo and he wanted to listen to a match on the radio. As the radio quality was weak, we had to finally stop and follow the broadcasting on the roadside in the desert. However, the only program Bill could find was in Afrikaans. Thus apart from the players’ names and the score, he did not understand a single word. An amazing fact about Bill was that, although he was a forceful speaker, loving to use and to play with the English language, he was completely ignorant about other languages. So he never learned Afrikaans and in other countries, other people had to cope with translations.

But Bill had encyclopedic knowledge of the natural sciences in general, and he could instantly give a lecture about southern African zoology, botany, or geology. He was easily connecting all this different knowledge into a broader, comprehensive framework and thereby developing new questions and ideas. This ability to communicate new or complex knowledge made him a very stimulating academic teacher, something which was certainly was one of the reasons why he was so popular on the National Geographic tours he was guiding in his later years. His non-protective way of openly sharing data and ideas, as well as critically and without any mercy dissecting project ideas, hopefully remains a model to all the many students and colleagues with whom he was communicating his entire life. Many of his ideas and projects now remain to be finished by others, most prominently the revision of the ‘bible’ *Bill Branch’s Field Guide to Snakes and other Reptiles of Southern Africa*, and the description of dozens of new reptile species he had already collected and deposited in the herpetological collection of the Port Elisabeth museum.

To me, Bill was much more than a good colleague,



Fig. 19. Bill Branch in a sad mood after his snake stick, proudly stolen from Aaron Bauer, broke while he tried to destroy an *Opuntia* in the Karoo, October 2012. Bill: “What an embarrassing death to a snake stick, killed by a plant” (Photo: Mark-Oliver Rödel).

although we did only meet occasionally. More often in recent years, he was a very good friend with whom I enjoyed discussing everything, not only science. However, the scientific discussions with him were a constant inspiration providing me with many, sometimes unusual ideas on how to interpret data or set up new projects. He introduced me to the African family of herpetologists and to Mozambique; and I am proud that I could introduce him to the West African herpetofauna and rainforests, and even convinced him (sometimes) that amphibians are not completely boring. For him, I would have even loved to see England take the World Cup in 2018. He died too early and in an unbelievably



Fig. 21. Bill Branch and Mark-Oliver Rödel with the holotype of the species named in their honour (Photo: Frank Tillack).



Fig. 20. Bill Branch and Mark-Oliver Rödel in July 2018 in Bill’s home in Port Elizabeth (Photo: Mark-Oliver Rödel).

cruel way. I am very happy that I could meet him one last time, shortly before his death in PE. He will always remain an unforgettable person and inspiration. His death is a great personal and scientific loss, and my thoughts are with his beloved wife Donvé and both their families.

Paul H. Skelton

Wild Bird Trust, National Geographic Okavango Wilderness Project

When the National Geographic Okavango Wilderness Project (NGOWP) was looking for key specialists to join them on expeditions into the unexplored highlands of Angola, Dr. Bill Branch was a first port of call. Bill was attracted into the NGOWP as an established authority of Southern African and Angolan herpetology, most especially the reptiles. He joined the founding 2015 NGOWP Expedition as part of the 'land party.' He also took part in the 2016 expedition, joining it after first enjoying an extensive journey through the escarpment reaches of Angola in the company of Dr. Pedro Vaz Pinto. Prior to this, he had visited Angola on a number of occasions, collecting and adding significantly to the herpetofaunal knowledge of the country. His collecting antics often drew curious onlookers, mostly children, who would marvel at what wonders he would bring forth from the ponds, rocks, and crevices. More significantly he both encouraged and actively mentored younger herpetologists currently active in Angola. These expeditions have resulted in several potential new species, a number of new species for Angola, and range extensions of many others.

Bill was an old friend and colleague of some of us. I personally met and knew Bill soon after he arrived in South Africa, and was working for the Atomic Energy Corporation outside Pretoria. On joining the Port Elizabeth Museum, we became good friends and he



Fig. 22. Bill Branch in Angola looking for frogs (Photo: Alex Paullin).

joined me on at least two interesting expeditions that I arranged primarily for fish sampling—one was along the Lower Orange River, and the other was to Lesotho in 1988. Bill had a 'nose' for reptiles. I remember vividly how he would relish the refreshment breaks on the expedition as we travelled north across the karoo, in order to sniff around the rocky kopjes and outcrops along the way. He inevitably returned with a clutch of reptiles in hand, all of which were unerringly shot with an elastic band from the hand. And then there was the spitting cobra, *Naja nigricollis woodii*, he caught by the tail on crossing the road, after screeching to a halt and leaping forth from the vehicle. As viewed from the vehicle, behind it was an energetic spectacle in madness—born out on arrival to realise that Bill had been spat in the eyes by the enraged reptile and, whilst he was blinded and in agony, was desperately directing his non-herpetological colleagues in the niceties of bringing a canvass bag to bear so he could insert the writhing beast. Needless to say, he succeeded and managed to wash his eyes out before he was permanently damaged. On the Lesotho trip, Bill was his amazing self and not only displayed his fly-fishing skills that I never knew he had, but also showed me the cryptic, super-jawed, Maluti River Frog (*Amieta vertebralis*) in its natural habitat. His calm demeanour, bubbling humour, and all-round knowledge in the field was always refreshing. Simply put, Bill was a pleasure to have around. His scientific productivity and achievements are of a top order. His passing was a great loss to our project and to the community at large.

Aaron M. Bauer

Villanova University, USA

I met Bill in 1987, during my first trip to South Africa. I had met with Alan Channing in San Francisco and he had given me a list of all of the critical herpetologists and institutions to visit in South Africa. After visits to Wulf Haacke at the then Transvaal Museum and to FitzSimons Snake Park in Durban, I made my way to Port Elizabeth via Cradock. I phoned Bill on the way (from a post office, remember no cell phones?) and he suggested that my field assistant and I stay at the camping ground on



Fig. 23. Bill Branch in Angola looking for tadpoles (Photo: Alex Paullin).

Brookes Hill. A gale was blowing and we were soaked to the bone, but the next morning Bill kindly showed me around the Museum complex. Over the next day or two he took me *Bradypodion* hunting in Happy Valley, just down Beach Road from the Museum, showed me the introduced *Lygodactylus capensis* on the guard rails along the roads, and sent me off to Schoenmakerskop to look for *Acontias meleagris*, *Homoroselaps lacteus*, and other reptiles. Like everyone I met on that first trip, Bill was a critical contact if I was intending to start working in South Africa. By 1989, I was coming regularly, sometime



Fig. 24. Bill Branch in Angola with a dead on the road Vine Snake (Photo: Alex Paullin).



Fig. 25. “Uncle Bill” enjoying the adoration of the masses (Marius Burger and Krystal Tolley) at the H.A.A. meeting in Cape Town in 2011 (Photo: Aaron Bauer).

two or three times a year, and more often than not Bill and I would go to the field together, starting a 30 year personal and professional collaboration that influenced all of my work in Africa as a whole.

I have very many fond memories of Bill. One was a 1990 trip to northern Namibia. I picked up Bill and drove with him and two of my students to a farm in Kamanjab. We stayed with the farm managers and had a wonderful time. The collecting was spectacular and mostly new to both me and Bill, who had not spent much time in Namibia before this. Every day we found additional species, in the end nearly 50 species on the farm alone, and more between Kamanjab and Palmwag. Bill had to leave before me and on his last night, after weeks of the best warthog and gemsbok, we were promised “something special,” which turned out to be a very old and very gamey goat! The next day Bill and I left the students and drove straight through to P.E. with only a short stop for a nap. Our only music in the car was The Greatest Hits of Elton John. On the trip we really got to know one another, and we both got so sick of Elton John that we couldn’t listen to his music for years.

Other fond memories are of our multi-year projects in the Little Karoo and later the Richtersveld. In those days there were very few visitors to the Richtersveld, and Bill and I both enjoyed the solitude of the park, evenings by the fire along the Orange River, and finding two *Bitis xeropaga* only meters away from one another. I can also mention a magical trip to the Kaokoveld along with Johan Marais and my Villanova colleague, Todd Jackman. We were in the bed of the Munutum River and all of a sudden we were surrounded by a herd of giraffe. Even Bill, always ready for the good photo opportunity, was temporarily awestruck by the scene. I also spent many memorable weeks with Bill in the States. One trip was to the South Carolina coast just after a hurricane. Despite some serious close calls with disaster, the loss of one of Bill’s cameras, and hundreds of mosquito bites, Bill was pleased to catch a baby alligator and to have

had the chance to be in the field with Whit Gibbons, a great herpetologist and ecologist, and an author whose writings Bill admired. On another trip, we drove 10,000 km from coast to coast and back in the US with my students and postdocs. At 3,700 m we saw a herd of elk and Bill managed to get most of his body outside of our moving van to get the perfect shot. I think all of these fond memories are united by the common theme of sharing with Bill the feeling of how lucky we are to have a vocation we love and that lets us enjoy spectacular animals in amazing places in the company of our friends.

Bill was the face of South African herpetology, indeed of African herpetology. His interests were wide-ranging and he had a mind for details when it came to all things herpetological. He was also a master naturalist who knew his birds and his plants, as well as the history of natural history exploration in Africa. He was also down-to-earth. Even the most novice of herpetologists was welcome to call him Bill, not Dr. Branch. Although he could, and often did, go on for hours about something in a quite serious tone, anyone who spent much time with Bill knew that he had a wicked sense of humor, and conversations with him could swing between debates about the International Code of Zoological Nomenclature one minute to a hectic exchange of friendly insults the next. That he was known as “Uncle Bill” to many speaks volumes about how comfortable we all felt with Bill. My relationship with him was somewhat different. Years ago I told Bill that I thought of him as the older brother I never had and indeed, the last words Bill spoke to me were “Be well, little brother.” We are all both better herpetologists and better people for having known Bill.

Stephen Spawls

Although Bill and I had corresponded since the early 1980’s, we didn’t meet until 1987, when Bill drove up to Botswana and stayed a few days with us at Moeding College, Otse. It was an exciting visit. Bill came in a white windowless Volkswagen Kombi, which was the same type of vehicle that had been used by the South Africa Defence Force on their 1985 raid into Botswana. Consequently, the Botswana security forces had tracked the vehicle, and as Bill drove out of our college he was stopped by the soldiers, who went through the vehicle. Finding nothing, the military concluded that Bill had cached his weapons at my house which was then searched! After this inauspicious start, my wife and I subsequently stayed with Bill in Port Elizabeth. We went on an amazing safari, to the Addo Elephant National Park, to Graaff-Reinet, and thence into the Karoo, where we stayed at the Karoo National Park headquarters with Bill’s friend, the warden Harold Braack. We returned via the Swartberg and Oudtshoorn.

Being in the field with Bill in some of his favourite country was an amazing experience; he knew the land, the customs, and the animals, and gave freely of his expertise. We found many spectacular species that were totally

new to me, including Hewitt's Ghost Frog, *Heleophryne hewitti*, the Giant Ground Gecko, *Chondrodactylus angulifer*, and the Blue-spotted Girdled Lizard, *Ninurta coeruleopunctatus*. But we weren't lucky all the time. At one point Bill and I drove for several hours at night on the tarred road near Beaufort West, hoping to find a Horned Adder, *Bitis caudalis*; but we saw virtually nothing. As we returned to the park, near midnight, there on the road was a snake, and we leapt out with great excitement but it turned out to be only a Herald Snake, *Crotaphopeltis hotamboeia*. One morning Bill and I were pursuing a sand lizard, *Pedioplanis*, which was sheltering in a clump of bush. As it appeared near my feet I dove at it but missed, and it fled to another clump. Bill clapped his hands on his head. 'It's obvious you've always collected by yourself', he said exasperatedly, 'you should have just shuffled it towards me, not leapt at it without telling me.'

On that trip, we also learnt of each other's shared enthusiasm for bird-watching. As we drove across country, Bill directed me to a side road. 'I've got a surprise for you,' he told me, as we took the diversion. We went a few miles and then Bill told me to pull up and get my binoculars; and there, in a grassy area below the road, were a pair of Blue Cranes, the first I had ever seen.

In 1991, I went to work in Ethiopia, and Bill wrote to me in 1992, suggesting we might work together on a book on Africa's dangerous snakes. Blandford Press showed interest in the project, and in 1993 Bill came and spent a few weeks with us in Ethiopia, doing field work and working on the book. We made several field trips, one was to the highlands east of the Rift Valley, to a town called Dodolla where a specimen of *Bitis parviocula*, the spectacular Ethiopian Mountain Viper, had been collected, still the only specimen known from east of the rift valley. As we ascended the rift valley wall, up through dense broad-leaved forest, we became increasingly excited; this looked like *Bitis parviocula* country. Then as we approached Dodolla, we emerged on the plateau, and found ourselves on a vast open grassland, as bald as a billiard table. Bill sighed and looked at me. 'Listen, matey' he said (Bill and I were both born in North London, he at Finsbury Park, me in Muswell Hill, and sometimes in the field we were just two Londoners together), 'we're looking for a forest viper, and as far as habitat goes, we've just gone from the sublime to the ridiculous.' But that day we did find some spectacular frogs, including *Paracassina kounhiensis*, Mocquard's Mountain Kassina.

The following week, down in Awash National Park, we had some remarkable luck; in one afternoon and evening we got a North-east African Carpet Viper (*Echis pyramidum*) under a rock right outside our room, on the road in the dark we found a Kenya Sand Boa and two species of egg-eater, and as we drove back to the lodge, we caught a huge *Atractaspis fallax* on the road, an adventure that Bill described as being 'like trying to subdue a spiked manhole cover.' At Lake Langano, we

caught a small Egyptian Cobra (*Naja haje*) on the road. Back in Addis Ababa, I was teaching one morning and Bill worked in the garden, creating a small set on top of a rather nice garden table to photograph the cobra. Unfortunately, he incorporated several biggish rocks in the set and in moving these around, he managed to thoroughly gouge the polished surface of the table. My wife went ballistic, but Bill turned on the charm and managed to persuade her that it was all part of the great scientific endeavour, and he took us out for a meal as well. The following day I found Bill crawling around in the canna lilies when I got home; one of the frogs he was photographing had sprung into the flowerbed and escaped.

We didn't always get on well. Bill had a very relaxed attitude towards deadlines, and often preferred to go into the field rather than knuckle down. He once told me how his publishers (Struik) 'had flown him to Cape Town' to finish his field guide, and a fellow herpetologist, who overheard this, said 'What you mean, Bill, is that Struik made you fly there, sat you down in their offices and said you weren't leaving until you got it finished.' Bill laughed and admitted it; and in one of his books he thanks his editor for 'making ridiculous deadlines seem acceptable.' Our work on the *Dangerous Snakes of Africa* book was complicated. Bill was in South Africa, I was in Ethiopia, and there was no e-mail in those days. We used to send stuff to each other by courier. As the deadline for the delivery of the manuscript approached, Bill had a lot of the snakebite stuff still to do and wasn't getting it done. With two weeks to go and the publishers muttering angrily about penalty clauses (the production was catalogued, and tied into a publicity/release schedule), I rang Port Elizabeth to be told that 'Dr. Branch had left on an extended safari to Zambia, and would not be back for a few weeks.' In a panic, I managed to get hold of Dr. Colin Tilbury, who stepped into the breach and wrote virtually all the snakebite stuff in short order. The manuscript went in on time, but it led to a furious row between Bill and I over the order of our names on the cover. But eventually we got over it, and in fact in 2017, we agreed to do a revision of the *Dangerous Snakes* book.

The last time I met Bill was in 2014, in Bagamoyo, Tanzania, where we were part of a team assessing Tanzania's reptile biodiversity. We went out one evening and I climbed a tree to catch a sleeping Spotted Bush Snake. Bill watched thoughtfully. 'I'm past climbing trees', he told me. 'In fact, I'm past climbing over anything. Last time I was in Namibia with Aaron Bauer, a lizard ran under quite a low fence and neither Aaron nor I could get over it.' He laughed, 'It's my fondness for prawn curry.' On that conference, Bill talked with great enthusiasm of a projected book. 'I really want to do a big book' he told me, 'covering the natural history of Africa's snakes, along the lines of Harry Greene's book.' He showed me some ideas and pictures on his computer; his ideas were mind-stretching and holistic; he saw



Fig. 26. Bill Branch photographing some lilies at Lake Langanao, Ethiopia (Photo: Steven Spawls).

the snakes in the landscape as part of the interlocking whole ecosystem, and his accompanying pictures were, as always with Bill's photographs, spectacular. Nobody else has photographed the African herpetofauna like Bill.

We started work on the revision of the *Dangerous Snakes* book in early 2017. Bill sent me some draft material, a list of important references (he had an encyclopaedic knowledge of the literature on African reptiles), and a stunning portfolio of pictures. But in late 2017, Bill cautiously wrote to tell me he was having mobility issues, and wasn't sure how this might affect our project. And in early 2018, to my shock, I heard from Bill that he had been diagnosed with motor neurone disease. But he remained full of optimism; and said that he fully intended to do his bit; cheerfully pointing out that Stephen Hawking had lasted many years with the same affliction. But it was not to be. The disease quickly took hold. Tragically, Bill died on the 14th October 2018. His untimely death is a major loss to African herpetology. And I hope that our book, which should be published in mid-2020, will be a suitable monument to Bill. Few herpetologists have reached both the public and their fellow scientists with such verve and accuracy as Bill did.



Fig. 28. Bill Branch and others at Bagamoyo, Tanzania, in 2014.



Fig. 27. Bill Branch admiring an old tank near Dodolla, Ethiopia (Photo: Steven Spawls).

Andrew Turner

CapeNature, Western Cape, South Africa

I first met Bill Branch at an HAA meeting, I think the Stellenbosch meeting of 1998 or thereabouts. He was the top South African herpetologist in my mind because of his comprehensive treatment of the reptiles of the region (his lesser interest in amphibians did not bother me, as his emphasis on the snakes more than made up for this). He was always interested in other people's experiences, especially regarding observations on distributional occurrence, and encouraged documenting this valuable form of data. Knowing that someone like Bill, who came from a rather different background, could switch to making a career of herpetology—and a rather exciting and enjoyable career at that—was a great inspiration for me to continue my professional herpetological interest. His photography was also inspirational and keeps me (and many others) clicking away.

Bill was a great raconteur, and his stories of herping gone wrong were particularly amusing. One story in particular, although I don't remember that exact mechanics of it, involved Bill trying to catch a *Cordylus* under a rock that was being lifted by a colleague. (I shall not mention his name but he did have an extensive snake collection at one point). Bill shoots his right hand under the rock to catch the *Cordylus* but notices at the same time there is a second *Cordylus* under the rock so shoots in his left hand too, catching both of them. But then a third *Cordylus* runs out from under the rock and said colleague catches that one by dropping the rock on both Bills hands!

Bill really set the scene for getting the full picture of South African herpetological diversity, and did a good job of placing this in an African and global context. He travelled widely and shared his great photographs, and was always wondering how the various species fitted together. His fondness for the small adders was totally understandable, and he did a good job of discerning their subtle (and probably recent) divergence and highlighting the need for conservation of several of these species.



Fig. 29. Bill doing what he did best: geeing everyone up to maximise income from the HAA auction! (Photo: Andrew Turner).

Ernst H.W. Baard

CapeNature, Western Cape, South Africa

My career as a herpetologist with CapeNature (then Cape Department of Nature and Environmental Conservation) started in January 1983. My first task was to sort out and process a few thousand specimens of frogs, lizards, snakes, and tortoises collected and collated by my predecessor, John Comrie Greig (Greig and Burdett, 1976. *Patterns in the Distribution of Southern African Terrestrial Tortoises*) and, at the time, colleagues, Richard Boycott and Atherton de Villiers.

After writing (yes, there were no emails those years) to the curator of the Port Elizabeth Museum, Dr. Bill Branch, and the curator of herpetology at the South African Museum in Cape Town, Dr. Geoff McLachlan, about depositing the specimens (roughly divided into the Western and Eastern Cape), we got positive responses from both curators. Atherton and myself proceeded, and we completed the task of sending, among others, the whole Greig and Burdett wet and dry tortoise collection, and several hundred “eastern” Cape lizards, snakes, and frogs to Bill at the Port Elizabeth Museum. Bill’s epic paper on the lizards of the Cape Province (Branch, William. 1981. *An Annotated Checklist of the Lizards of the Cape Province*) made a huge impact on my knowledge of the lizards of the Cape, and together with FitzSimons 1943 (*Lizards of South Africa*) guided us through the process.

An incident that stood out during this time, was the discovery in the Jonkershoek collection of an unidentified many-spotted lizard in a small bottle, collected in 1973. It was beautifully preserved and, fortunately, with a geographical location in the Groot Winterhoek Mountains down to seconds South and East (this was before GPS). It took me a few days using FitzSimons 1943 to identify the lizard as “*Lacerta*” *australis*, and we were very excited about this discovery. One unsuccessful collection trip to the locality (by Boycott, De Villiers, and Baard) was undertaken in February 1983, followed by Atherton and I managing to collect two more specimens

at the same locality in April 1983. Imagine our joy, since these were as far as we could establish, only the 6th and 7th specimens known to herpetology of this “elusive” species. This information was shared with Bill and he encouraged us to publish a note which we promptly did, asking him to co-author (De Villiers, Baard and Branch. 1983. ‘*Lacerta*’ *australis*: additional material). Bill was always supportive of any further investigations and readily responded to queries on the herpetological collection.

In later years, Bill got back to us and was very excited about some of the tortoise specimens we sent him, including some of the largest individuals of some species he had encountered. He then published a short note in the Journal at the time, honouring Atherton and I with co-authorship (Branch, Baard and De Villiers. 1990. *Some exceptionally large Southern African chelonians*).

I only met Bill for the first time at my first HAA Conference in Stellenbosch a year or two later, and was really honoured to make his acquaintance. His paper on angulate tortoise ecology in the Eastern Cape (Branch 1984. *Preliminary observations on the ecology of the Angulate Tortoise*) had a huge impact on my career, since this paper shaped my thoughts and guided my research and attempts at understanding the ecology of the geometric tortoise of the Western Cape; having completed my research in 1990. For a young herpetologist like me at the time, it was almost natural to think: *What would Bill do in this case?* or *How would Bill approach this topic?*

Bill’s astonishing knowledge of lizards, tortoises and snakes, snake venom, snakebite, etc. was really something to behold, and few herpetologists could keep up with him. I fondly remember Bill at conferences communicating with all and sharing his knowledge. The best story I remember him telling, was about the evening in the veld around the fire. After Marius Burger latched a *Pseudocordylus* crag lizard to his (Marius’) earlobe, Bill kept on touching the lizard which wouldn’t let go of Marius’ ear; with the lizard biting down harder and harder, Bill spent an hour or so enjoying Marius’ agony and futile attempts to get the lizard to let go of his ear!

William R. (Bill) Branch was a legend of his generation and time. Not only was he a brilliant scientist, excellent herpetologist, and I believe, a great bird enthusiast, but also somebody one could look up to. His contribution to South African and global herpetology will go down in history as exceptional, ground-breaking, and outstanding, and will stand the test of time like with other greats; FitzSimons, Broadley, etc. His contribution to the written and peer-reviewed herpetological science and popular literature is unsurpassed, and it is my honour to have known him.

Amber Jackson

Cape Town, South Africa

Uncle Bill’s Bible was a well-used field guide by the time I met the man himself. I had even sent him a few



Fig. 30. DNA sampling of Spek's Hinged Tortoise, *Kinixys spekii* (Photo: Amber Jackson).

specimens of *Leptotyphlops* years before when I was a student. I finally met Bill as an awe struck herpie requesting he sign my copy of his field guide before a very serious meeting at my new place of work. He wrote: "Wow, Uncle Bills bible!!! 167 species out of date, but what else is there." Out of nowhere he then spouted a lecture about the Galapagos and island biogeography, and held up the meeting for 20 minutes in the process. All eyes on me, I left with my signed copy and some knowledge I never requested but was all the better for knowing. Little did I know, our first meeting was an accurate precursor for the years that followed. Thanks to numerous development EIA's Bill and I travelled to Lesotho, Augrabies, and Mozambique (multiple times), with me always as his self-proclaimed assistant.

One of my favourite memories with Bill is lying in the dark, on a rocky shelf at the top of the Augrabies paleo falls, staring at the stars and waiting for the geckos to come back out after our disturbance. The stars were incredible! I later caught him a *Pachydactylus atorquatus*, without breaking the skin, and received an exclamation of 'I could kiss you.' He didn't, and ran off with his prize. At the time, I was naïvely more excited that it meant we could go to bed before our dawn wakeup call in four hours. Bill, 40 years my senior, put me to shame with his energy levels.

We got along at first because I was eager to learn, and he was eager to teach. Then one day, I called him a bastard for one of the anti-feminist comments he used to purposefully provoke me with, to which he laughed,



Fig. 31. Bill reading science in 45 °C heat (Photo: Amber Jackson).

sighed, and said "finally!" All pretenses over with, we were then friends.

Most of my time with Bill was spent learning, so much so that my brain stopped being able to absorb any more information and saturated by the end of the long day. The knowledge he possessed was impressive, diverse, and felt insurmountable. He taught me plenty about herpetology and science in general. His enthusiasm was contagious and witnessing his studiousness in the field was impressive, with his daily diary and specimen processing. But one thing that stands out is the things he probably never intended to teach me, for example: How being reserved doesn't have to impact negatively on your life, how euthanizing something as cute as a bush baby can make a huge contribution to science and someone's career, or how LED lights can make fresh produce more appealing. How just because your life starts in one place doesn't mean you have to stay there. How you can be a jack of all trades and a master of one. How you can make mistakes. That apologies are important. How careers can be diverse and often unfold. That one of the biggest joys is to love, freely and openly.

To the man that "*was by turns (and somehow all at once) relaxed, intense, sincere, self-mocking, modest, confident, serious, and funny.*" Kim Stanley Robinson

To the man that could make an economist understand biodiversity by using economic terms.

To the man that could answer the question: "Is a penguin a fish or a bird?" politely and honestly.

To the man that could provoke an entire lecture with a simple question: 'What's the odd one out?'

To the man that believed in love, in science and in the unknown.

To the man that studied cancer but fell in love with a cobra while fishing.

To kind, funny, and sometimes forgetful 'Bum in the Butter' Bill.

I think about you often, your teachings, your adventures,

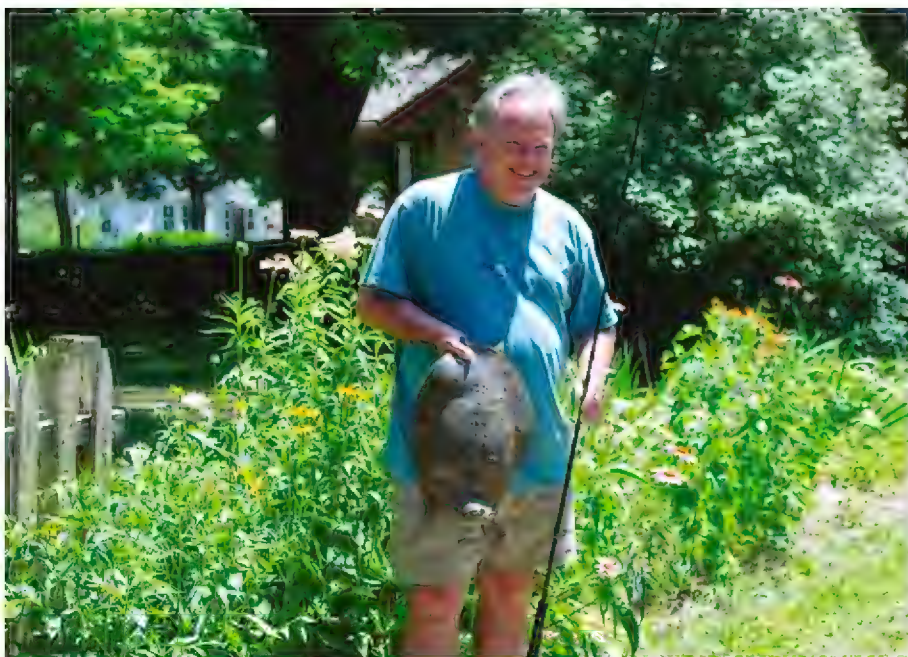


Fig. 32. Bill with a shoftshell terrapin he caught (Photo: Craig Weatherby).

our last day together, and all the days before then. I couldn't have asked for a better mentor, teacher, and dearest friend. I will treasure you always. Thank you for believing in me.

Margaretha Hofmeyr

University of the Western Cape, South Africa

I first met Bill Branch in October 2000, when my colleague Alan Channing invited him along on a field trip to Namibia for the UWC Zoology Honours students. I had most students with me in my husband's Kombi Synchro, while Bill was a passenger in Alan's Land Rover. We stopped at Springbok's Caravan Park for the first night, where I booked accommodation in chalets. Because Bill joined the party at the last minute, I knew there would not be a bed for him and had some concerns about sleeping arrangements. For one or other reason, perhaps because Bill was idolised by all herpetologists, I expected him to be rather arrogant, but he quickly won me over when he made a bed for himself in the trailer Alan took along. The trailer was quite short, but so was Bill; fortunately the trailer was rather wide, because so was Bill. The sight of him surfacing the next morning from his trailer bed will always stay with me. Yes, he might have been arrogant at times, but he was always a great sport, and teased the students to distraction on this trip.

I always feel dishonest calling myself a herpetologist, because my field of expertise is restricted to tortoises and terrapins. Yet, on this trip, as herpetologists do, we went on night drives to look for herps (never tortoises) on the roads. One of the nights while staying at Klein Aus, everybody squeezed into my Kombi to search for exciting things on the roads. While driving through a narrow stretch of road between two fences, a springbok ram materialised in the road before my husband's car. I switched the main lights off within seconds, but it was still too late. The springbok ran straight into the Kombi, broke his neck, and put quite a dent into the front of the car. All the girls were crying but we had to deal with the situation. Alan and Bill dragged the springbok out of the road and then we had the unfortunate task of driving to the owner's house to report the incident. His only

reaction was that it was the only springbok he had on the farm. This was an unpleasant experience for all of us but also created a bond, because Bill mentioned it many times to me in ensuing years.

At conferences, I would get annoyed with fellow herpetologists for teasing Bill about his lisp, yet, he always laughed at their jokes. To me, the ability to laugh at yourself reflects true character, and Bill had that. I have many fond memories of Bill and always regarded him as the ultimate herpetologist and naturalist in South Africa. His expertise stretched so much wider than reptiles and amphibians. He may not have been an expert on every animal or herp group, but his knowledge was astounding. He was also willing to share his expertise and helped many young scientists to find their way. I may not be described as a young scientist, but when I switched from large mammals to tortoises, Bill knew much more than I did, and he was willing to share. Over the years we co-authored several papers and it was always a pleasure to work with him in a professional capacity. South Africa, Africa and the World are now deprived of one of their top intellectuals, and an exceptional person—we salute you Bill.

Jens Reissig

Ultimate Creatures, Gauteng, South Africa

The first time I met Bill was during a high school field trip to northern KwaZulu-Natal around the year 2000. At that stage, I was rather shy and having had a very keen interest in reptiles since my early childhood I of course knew exactly who he was, however never made any contact with him. Many years had passed until I crossed paths with him again at the Herpetological Association of Africa's Conference at the National Zoological Gardens in Pretoria, South Africa. From this point on, we stayed in contact and he was always willing to help wherever he could. Unfortunately, he was extremely busy while I was compiling my book on the Girdled Lizards and their Relatives in 2013 and 2014, so that he was not able to assist me with it in any way. He did however end up reviewing the book for me. The book review ended up being published in *Herpetological Review*, 2015, 46(2): 1–7.

After having received the tragic news of Bill's diagnosis, I decided to go and visit him at his home in Port Elizabeth on the 20th of April 2018. Even though one could see that he was battling his illness, he still tried to be upbeat about life and could not stop talking about reptiles and a (to me) hidden passion of his, Orchids. We sat for hours on his patio talking about various reptile projects, his Orchid collection, birding, and some of his many field trips into Africa. After spending quite some time with us, one could see that he was tiring and we decided to say our goodbyes and I left. It was the best day I had ever spent with him. My favourite email I have ever received from him was received on the 12th of June 2015 and stated: "Dear Jens. Here are the proofs of my review of your excellent book which should appear in



Fig. 33. 2017 Herpetological Association of Africa's Conference in Bonamanzi, South Africa. From left to right: Werner Conradie, Tyrone Ping, Prof. William Branch, Luke Verburgt, Dr. Michael Bates, Johan Marais, Prof. Graham Alexander, Prof. Aaron Bauer, Jens Reissig, Coleen Tiedemann, Dr. Colin Tilbury, and Dr. Victor Loehr (Photo: Andre Coetzer).

Herp Review this month. Hope you're happy with it and all goes well. Best wishes. Bill"

Professor W.R. Branch, your passing has left a massive hole in so many people's lives and in African Herpetology as a whole. Your knowledge and sense of humour will be greatly missed by anyone who ever had the privilege to know you and who's life you may have affected in some way or another. Africa has lost two great herpetologists way too soon and in relatively quick succession. Till we meet again!

Harold Braack *South Africa*

Bill Branch first came into my life, I think, in 1974, at an HAA conference held at Skukuza in the Kruger National Park (KNP). It might have been the first such get-together. At that time, I was doing the herpetology survey of the KNP, so it was indeed fortuitous to meet Bill Branch, as well as Don Broadley and Carl Gans.

In 1976, I was transferred to the Bontebok National Park and so I started species surveys and checklists. I wanted to know what it was that I was supposed to be looking for, and so Bill and I started working together through various National Parks and adjacent areas. But it was not only herps. We looked at succulent plants and birds as well.

What Bill gave me was the confidence to do the surveys. In him, I had a partner with whom I could share my passion for conservation and protection of all the inhabitants of those areas. He was totally enthusiastic and this rubbed off on us all. Above all, he was a good friend.

I have many fond memories of Bill, but share only a few here.

The pepper ticks at Addo National Park were a vast irritation to Bill. We picked them up every time we ventured on a collecting trip. He hated them. I wiped them off with paraffin. But Bill had a different solution.

"Oh no," he said. "I go home. I get undressed completely then lie down in the nude on the kitchen counter. My family has to pick them off."

Bill Duellman and Bill Branch stayed with us in the Karoo for several days. To separate the two, we called Duellman "Bill" and Branch "Billikins." Bill B. was not totally enamoured with the solution.

Bill and I did a night road survey in the Richtersveld. After several hours we arrived at Paradyskloof. We lay down flat on our backs for a while counting passing satellites, then later scratching among the rocks where we saw the largest *Hadogenes* that either of us had seen. Then Bill went to the little pool to find a *Strongylopus springbokensis*. Ka-splash, splash. Bill fell in the pond. He sat huddled in the bakkie on the way home.

Spending a long, long time trying to catch a lizard in Richtersveld, Bill suddenly ran back to the bakkie. Out he came with a revolver loaded with dust shot. "Ka-boom!" he shot the thing—we had our specimen.

He had excellent repartee and a quick lucid mind. How many of us remember his response during a frog meeting at Stellenbosch? The chair said we should be democratic in the course of the meeting. Bill's immediate response "Thank You, Mr. Mugabe."

Bill also enjoyed fishing, especially for carp. We spent some time along the banks of the Orange and Breede Rivers doing just that. Didn't catch much, but those were relaxing times.

I best remember Bill as a man who was a dear friend. To all of us, he revealed the treasure chest of our vast herpetological wealth—and, more, he opened it up for us to see and explore. He followed his passion with a radiant glee which he passed on to us.

Bill, my friend, I salute you for being a friend, a guide, and an explorer who found and revealed.

Atherton de Villiers *CapeNature, Western Cape, South Africa*

I have good memories of Bill that date back to when his career in herpetology started at Port Elizabeth Museum, and have always admired his enthusiasm and vast knowledge of reptiles and amphibians. It is well known that one of his greatest achievements was his *Field Guide to the Snakes and other Reptiles of Southern Africa*. This landmark publication opened up the world of reptiles to countless numbers of people, and it was a pleasure to contribute information and images for one of the most important herpetological publications in southern Africa. I share with you all the huge loss of Bill to herpetology, biodiversity conservation, and life in general.

Marius Burger *North-West University, South Africa*

Try as I may, I just can't seem to pinpoint the precise memory of actually meeting Bill for the first time. I'm quite shocked by this realisation. I presume that it was sometime during 1987 when I was a young (20 y/o)



Fig. 34. Ozzy Osbourne meets Elvis Presley. My all-time favourite photo of Bill and I (Photo: John Measey).

nature conservation student in Grahamstown, and I vaguely remember something about visiting him at his office at Port Elizabeth Museum (PEM). The only definite lead that I have to go on is a specimen of Karoo (ex-Namaqua) Plated Lizard (*Gerrhosaurus typicus*) that I collected in November 1987 on the Karoo Nature Reserve in Graaff-Reinet. At the time, this record represented an eastern distribution range extension of 211 km. Whoopee! I would have hurriedly taken the specimen to Bill at PEM, with my tail wagging in excitement. Yes, what a joy it always was to make some sort of new herpetological discovery that even The Bill Branch would find somewhat noteworthy. And so it came to pass that my first ‘scientific publication’ was a short note in the *Journal of the Herpetological Association of Africa* (Burger 1988). The truth be known, Bill actually wrote the damn thing. But this was my official introduction to the Herpetological Association of Africa, and it marks the approximate start of a very lekker 30-year friendship with Bill.

An article published in *Zootaxa* on 24 October 2018 demonstrated that the African Slender-snouted Crocodile (*Mecistops cataphractus*) is in fact comprised of two superficially cryptic species, and thus *M. leptorhynchus* from Central Africa was resurrected as a valid species (Shirley et al. 2018). The first thought that crossed my mind when I read this paper was “*Fok, Bill didn’t get to see this!*”, because Bill had died ten days earlier. Bill would have loved the news that *Mecistops* is monotypic no more, and perhaps (probably) he even knew that this was in the pipeline. Fast-forward nine months to July 2019 (i.e., right now as I’m writing this), and I’m still experiencing *Where-TF-is-Bill* moments on an almost daily basis. Bill was my Google Herps. Whenever I needed photos of far-flung African reptiles to be identified, the oh-so-convenient Google Herps would



Fig. 35. Bill emerging from swamp in Gabon (2002) after checking funnel traps that he had set with the hope of catching an African *Parachanna* (Photo: Marius Burger).

usually be my first check. When the complexities of taxonomy would bewilder my brain, Bill had the knack of explaining it in a way that I could sort of comprehend. And so for me, it is not only very sad, but also utterly inconvenient and totally *kak* that Bill died.

It always intrigued me that a Pom could arrive in Africa with some sort of medical doctorate degree, something to do with foetal rabbit liver metabolism and primary liver cancer, only to end up perusing a career of chasing reptiles and amphibians. Like, how the hell did that happen?! Anyway, it’s a good thing that it turned out the way it did. Well, so says I, because I have derived much joy and intellectual enrichment from the times hanging out with Bill. To South Africans, Bill was loved and respected as our local herp guru. He was of course also internationally renowned for his herpetological contributions, and the momentum that he built up over the decades will have him publishing papers for a long while after he clocked out.



Fig. 36. Bill receiving medical attention during a biodiversity survey of Loango National Park (2002). If I remember correctly, it had something to do with removing ticks from a place where the sun don’t shine (Photo: Carlton Ward Jr.).

This may be somewhat of a narcissistic trait of mine, but I like it that Bill liked me. I would often purposefully say and do socially objectionable stuff in Bill's presence, for the reward of his approval and appreciation of my crudeness. Now that I think about it, this kind of behaviour was probably akin to a son showing off in front of his father for attention and approval. In a book that collated a collection of interviews on how to become a herpetologist (Li Vigni 2013), Bill wrote the following: "*I also never miss a chance to be with Marius Burger, just to re-emphasize how sane I am.*" Whenever Bill acknowledged me in a publication, I would smile, and feel all warm and gushy inside for receiving his praises. In the acknowledgments section of *Tortoises, Terrapins & Turtles of Africa* (Branch 2008), Bill wrote: "*A special thanks to Marius Burger, whose tortoise photography is just too good.*" Well, just imagine the grin on my face for that bit of flattery. He then went on to say: "*...if he could only look after his camera lenses as well as his hair...*" in *The Dangerous Snakes of Africa* (Spawls and Branch 1995), and Bill included a thanks to a certain Marias (sic) Birger (sic) for companionship and advice. If that was indeed me that he was referring to, then I say ditto to that.

Whilst on a fieldtrip with Olivier Pauwels and Bill Branch in Gabon, the three of us shared a shipping container that was modified into a bedroom of sorts. I retired to bed late one evening, with Olivier and Bill giggling away like preteen girls. The reason for their hysterics was that they had planted a condom half-filled with Condensed Milk in my bed. How silly is that! Anyway, I never noticed said condom in my bed and managed to fall asleep in spite of the spurts and snorts of laughter. The next day whilst checking our trap arrays they told me of their really funny prank, all the while grinning from ear to ear as they awaited my reaction. Instead of shock and dismay, I replied with a calm reminder that a cleaning team was making our beds each morning and just imagine what their take would be on discovering this soggy item in one of our beds. I watched as their expressions gradually turned from smile to mild alarm, as the two of them slowly processed and realised the gravity of this scenario. Now it was my turn to laugh.

I'm not a spiritual kind of guy, and thus I won't be saying things like *R.I.P. old friend* or *check you on the other side*. But ja, Bill was for sure a significant component of my life. I am very chuffed to have had him as a friend.

Mike Raath

Director, Port Elizabeth Museum Complex (now Bayworld), 1987–1995

I first met Bill Branch in the early 1980s, when I was at Wits University as head of the Bernard Price Institute for Palaeontological Research. I had been invited by Prof. Brian Allanson of Rhodes University's Zoology Department to present a short course on the evolution of the Class Reptilia at my much loved Alma Mater.

At that early point in my career, I only knew of Bill by reputation, and had never met him personally. I felt flattered that he had taken the time and trouble to travel from Port Elizabeth to Grahamstown to listen to my ramblings. I little realised then that he and I would meet up again in a different context several years later, when I was fortunate enough to be appointed Director of the Port Elizabeth Museum Complex, as it was then called before it got its trendy current name of 'Bayworld.'

Bill was a much-respected member of the research staff of the Complex, having charge of one of the most comprehensive and important herpetological collections in the country, building on the solid legacy of its original founder, the legendary F.W. FitzSimons, almost a century before. He was one of the stars of our research team, regularly producing work that was published in some of the world's top peer-reviewed scientific journals. But in addition, he was a prolific writer of popular articles and books aimed at the general reader that spread his expert knowledge to a much wider general readership. I remember one envious member of our research staff calling him "the Naas Botha of our research team" in terms of earning brownie-points for research output (only those who know something about South African rugby in the 1980s will understand that comment!).

One of the things that defined Bill was his off-the-wall unconventionality. I remember how audiences at his various public presentations would shudder in shock, horror, and jaw-dropping disbelief when he demonstrated his go-to technique for distinguishing between identical sibling species of toads—by licking them! And, by Jove, it worked!

I respected Bill as a person and as a scientist. But to be candid, I have to say that he and I did not get on that well personally. He suffered neither fools nor administrators gladly, so as his director I guess I failed on both counts! But as a scientist committed to his discipline there was no faulting him. He was single-mindedly devoted to his collection and his research, often to be found in his lab or office over weekends or public holidays when most others on the staff were taking what they rightly regarded as a well-earned rest.

One particular Saturday morning remains starkly and darkly etched in my memory, when Bill received an urgent call in his lab mid-morning from the Snake Park. One of the Snake Handlers, Mr. Nimrod Mkalipi, had been bitten by a Puff Adder at the end of the daily live snake demonstration, and he was in dire distress. Bill dropped everything and rushed to Nimrod's side, administering antivenom and applying appropriate emergency first aid. Tragically, though, it was to no avail and Nimrod succumbed on the scene despite Bill's urgent, expert, and devoted efforts. Medical opinion afterwards held that it was anaphylactic shock that took Nimrod's life, and that nothing other than immediate on-site specialised medical intervention would have had any chance of preventing it. That event shocked us all. It is a dark memory that I



Fig. 37. Bill walking off into the early morning light to photograph some Welwitschias in south-western Angola in 2009 (Photo: Werner Conradie).

carry with me to this day, but I applaud Bill Branch for his swift reaction and his valiant and urgent attempts to save the life of a fellow staff member. We all had much to learn from that tragedy.

Werner Conradie

Port Elizabeth Museum, South Africa

The first time I became aware of Bill was when I attended my very first HAA conference at Bayworld in 2004, which he organised. I remember the occasion very clearly, as the only options available at the icebreaker were beer, and Coca-Cola. I was too shy to speak to him then—he was the famous Bill Branch and I was, after all, just a lowly student. I met Bill again at the 2006 HAA held at Potchefstroom, and this time I was on the organising committee. At this event I recall fondly Bill's talk on 'guts and gonads,' which of course went well over its allotted time. However, it wasn't until I finished university completely that we would have what would turn out to be a bit of a prophetic chance encounter. While on a December break, just before I would start a new job as a Physical Science high school teacher, me and my now wife walked into him while strolling around the museum. I introduced myself to him, finally having a moment in his direct eyesight after all this time, and with a curt nod paired with a brief "nice to meet you," he disappeared through the door to his lab and office. Never would I have guessed that less than six months after this, I will be sitting in front of him for an interview for the job of Assistant Herpetologist. I must have impressed Bill one way or the other (couldn't have been pure desperation), as two days later I received a call that I got the job. I finished my contract at the school as fast as I could, and with great excitement walked straight into the museum the very same day. Bill looked at me in utter shock and sent me home, saying I should come back in the New Year... I guess he wasn't prepared for my quick start!

For the first year at the museum I had to learn all the ropes. Now this is a very steep uphill battle for



Fig. 38. Bill holding a Meller's Chameleon (*Trioceros melleri*), looking radiant despite a very tiring hike during the summit of Mt. Mabu in 2009 (Photo: Werner Conradie).

an Afrikaans-speaking seun that knew his frogs, but no reptiles. Up to that point in time, the only reptile ever caught by me was a harmless Common Brown Water Snake. The first challenge I faced was getting into a conversation with Bill. Because of his British heritage, he mumbled a lot and this made me struggle to understand his pronunciation of scientific names. After many "conversations," I often went back to my office and paged through his field guide to prepare for the next engagement. Bill, however, never forced his reptilian inclinations on me. Once, he walked into my office and promptly asked me what I wanted to specialise in, to which my response was tadpoles. He dryly remarked that the only thing they are good for is fish bait. As it turns out, I never did work on tadpoles that much...

I went into the field with Bill for the first time as part of a multi-collaborative expedition to Angola in 2009. Bill didn't have to bring me along, he could have kept all the new places and specimens to himself, but I will ever be grateful as it was on that trip that I fully came to understand and realise what my responsibilities as a museum herpetologist include: New discoveries! I joined Bill on two more consecutive trips to Mount Mabu, Mozambique in 2009 and Lagoa Carumbo, Angola in 2011. It was at this stage that Bill assisted me with my first-ever species description, and just as I was starting to bask in the glow of his knowledge, he clearly thought he had trained me enough and turned off the light. It seemed I was on my own: Bill expected me to swim. It was up to

me to show him that I could. We wouldn't go on another field trip together again until 2015, again to Angola. By this time, Bill had retired, and I was keeping the fort on my own. During the trip, around the campfire one evening, Bill told me that he can now rest in peace, knowing the Port Elizabeth Museum herpetology collection is in good hands. Thank you, Bill.

I worked with Bill for more than ten years, but I only really started to get to truly know him and his family when he was unfortunately diagnosed with motor neuron disease. It was a devastating experience to see your mentor and friend fade away in front of your eyes. Bill was determined to follow his passion to the very end, and his determination was amazing to behold. Bill has taught me life lessons that I will cherish forever. He was truly a one-of-a-kind man. He is and will be missed.

Martin J. Whiting

Department of Biological Sciences, Macquarie University, Sydney, Australia

I first met Bill in 1994, just as I was about to start my Ph.D. working on flat lizards (*Platysaurus*). I was based at the Transvaal Museum in Pretoria, and he arrived to slice up snakes as part of a project on the ecology of African snakes with Rick Shine, Jonathan Webb, and Peter Harlow. Shortly after meeting him, he told me about the Augrabies flat lizard system, which ended up being the subject of my Ph.D. and many happy field seasons. I owed him a huge debt, without realising it at the time! And as it turned out, our discussions about flat lizards led to a collaboration that continued until his death.

Everyone that meets Bill is immediately struck by how warm and caring he is. It's hard to describe, but he had a personality that immediately drew you in. And I think that's why he had such an impact on so many people. He was particularly giving and helpful to young aspiring herpetologists, and I very much appreciated his friendship and advice as a young Ph.D. student fresh on the herpetological scene in South Africa. A few years into my Ph.D., he invited me on a field trip to a remote area of northern Mozambique to survey the vertebrates of the Moebase region, the site of a proposed titanium mine (sadly). I really appreciated this gesture, because he could have invited any number of far more qualified people! His son Tom was also on the trip, to survey birds. Little did I know that this would become such a memorable trip, and that I would have experiences I still talk about to this day. There is nothing like a field trip to really get to know someone, and that trip forged a lifelong friendship. With Bill, there was never a shortage of stimulating conversation on a wide range of topics beyond herpetology. His love for natural history was

infectious. The only thing Bill spoke about with more passion was his wife Donvé. While on that same field trip to Mozambique, Bill set out to find a clay pot that was representative of the region, to take back to Donvé. I should mention that Donvé is an award winning potter, so this was the perfect gift! Our fixer couldn't quite understand what a westerner would want with a clay pot, but we examined quite a few, before buying one from a surprised local villager. Actually, I also acquired one which has survived multiple moves in South Africa and a final move to Australia. (How could I not buy one after hearing Bill wax on about Donvé and her pottery!)

Bill was larger than life and made a huge impact on African herpetology. It's hard to accept that he's gone, but he will never be forgotten. He will certainly be missed by many. I am currently working on finishing a phylogeny and revision of the *Platysaurus* with Scott Keogh and Mitzy Pepper, a project that Bill and the late Don Broadley were both involved in, and he will certainly be in our thoughts as we put together the final touches.



Fig. 39. Bill and his son Tom while passing through a village during our 1997 Mozambique trip (scanned from a slide). To this day, that field trip ranks as one of my most memorable (Photo: Martin Whiting).



Fig. 40. Bill in action during our 1997 Mozambique trip [scanned from slides] (Photo: Martin Whiting).

Literature Cited

- Branch B. 2008. *Tortoises, Terrapins & Turtles of Africa*. Struik Publishers, Cape Town, South Africa. 128 p.
- Burger M. 1988. Geographical distribution: *Gerrhosaurus typicus*. *Journal of the Herpetological Association of Africa* 35: 36.
- Li Vigni F (Editor). 2013. *A Life for Reptiles and Amphibians, Volume 1*. Chimaira, Frankfurt, Germany. 495 p.
- Shirley MH, Carr AN, Nestler JH, Vliet KA, Brochu CA. 2018. Systematic revision of the living African Slender-snouted Crocodiles (*Mecistops* Gray, 1844). *Zootaxa* 4504(2): 151–193.
- Spawls S, Branch B. 1995. *The Dangerous Snakes of Africa*. Southern Book Publishers, Halfway House, Johannesburg, South Africa. 192 p.



Werner Conradie holds a Masters in Environmental Science (M.Env.Sc.) and has 12 years of experience with the southern African herpetofauna. His main research interests focus on the taxonomy, conservation, and ecology of amphibians and reptiles. Werner has published numerous principal and collaborative scientific papers, and has served on a number of conservation and scientific panels, including the Southern African Reptile and Amphibian Relisting Committees. Werner has undertaken research expeditions to various countries including Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Zambia, and Zimbabwe. He is currently the Curator of Herpetology at the Port Elizabeth Museum (Bayworld), South Africa.



Michael L. Grieneisen spent much of his childhood searching for and observing herps in the Appalachian Mountains of Pennsylvania, USA. He obtained a B.S. in Biology and Chemistry from Shippensburg University in Pennsylvania, and a Ph.D. in Biology from University of North Carolina, Chapel Hill, on a National Science Foundation graduate fellowship. Mike's Ph.D. and post-doc work (at University of Nevada, Reno) investigated the hormones that turn caterpillars into butterflies. Over the past 12 years at University of California, Davis, Mike has authored journal articles in fields as diverse as nanotechnology, climate change, biodiversity, scientometrics, environmental science, and reduced-risk pest control practices in California. Mike is a freelance editor, co-editor of *Amphibian & Reptile Conservation*, and he is compiling metadata for the theses and dissertations on amphibians and reptiles produced worldwide. The compilation currently includes over 54,000 theses, completed from 1803 to the present at institutions in well over 100 countries, and is expected to be made available sometime in 2020. He also has an extensive collection of world banknotes which feature herps in the design.



Craig L. Hassapakis is the Founder, Co-editor, and Publisher of the journal *Amphibian & Reptile Conservation* (official journal website: amphibian-reptile-conservation.org), which was founded in 1996, and a former editor of *FrogLog* (www.amphibians.org/froglog/). Craig has been an instructor (first grade through college), non-profit and governmental volunteer at Public Library of Science (PLOS), Co-group Facilitator, Genome Resources Working Group, IUCN/SSC Amphibian Specialist Group (ASG), and is a member of the IUCN/SSC Amphibian Specialist Group. His interests include biodiversity, evolution, systematics, phylogenetics, taxonomy, conservation, and behavior of amphibians and reptiles. Craig is instrumental in developing and establishing “*Amphibia Bank: A genome resource cryobank and network for amphibian species worldwide*.” His professional memberships include: *Society for the Study of Amphibian and Reptiles* (SSAR), *Herpetologists' League* (HL), *International Society for Biological and Environmental Repositories* (ISBER), and *International Society for the History and Bibliography of Herpetology* (ISHBH).









A herpetological survey of western Zambia

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Abstract.—A list of 60 species of amphibians and reptiles found during a six-week survey in western Zambia is presented. Two species of amphibians are newly reported for Zambia: *Amietia chapini* and an undescribed species of *Tomopterna*, previously known to occur in the Democratic Republic of Congo and in Namibia, respectively. Some of the material collected could not be confidently identified to species level because of the taxonomic complexity and uncertainty of some groups (e.g., *Phrynobatrachus*, *Ptychadena*), even with the use of DNA barcoding. This list is a small contribution to the growing knowledge of Zambian and African herpetology.

Keywords. Amphibians, barcode, checklist, reptiles, Southern Africa, undescribed species

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Introduction

Zambia is a landlocked southern African country considered part of the Zambesiaca area, which also includes Botswana, Malawi, Mozambique, parts of Namibia (Caprivi), and Zimbabwe (Poynton and Broadley 1991). Zambia is located on the main central African plateau where elevations range from 1,200 m to 1,500 m and the vegetation is dominated by *miombo* woodland (Phiri 2005).

Very little has been published on the herpetofauna of Zambia since the first then comprehensive reports from the early 1900's (see reviews in Haagner et al. 2000; Pietersen et al. 2017). Poynton and Broadley's compendium *Amphibia Zambesiaca* (Poynton and Broadley 1985a,b, 1987, 1988, 1991) and Channing (2001) reported on the distribution of Zambian amphibians, while Broadley (1971) presented an initial treatise of the reptiles and amphibians of Zambia, and Broadley et al. (2003) provided an updated atlas and field guide to the snakes of Zambia. Similarly, Haagner et al. (2000) and more recently Pietersen et al. (2017) have made important contributions to Zambian herpetology. Broadley (1991) presents a comprehensive list of reptiles and amphibians from the Mwinilunga District, northwestern Zambia, including records from museum collections dating from 1957. However, except from the extreme north-west (Hillwood Farm), the herpetofauna of western Zambia remains very poorly studied, with only a few regional checklists (e.g., Broadley 1991; Pietersen et al. 2017).

Currently, there are 189 species of reptiles recorded for Zambia according to The Reptile Database (Uetz et al. 2018) and 181 (two crocodile, 10 chelonian, 78 lizard, and 91 snake species) according to Pietersen et al. (2017). The number of amphibian species varies substantially according to different sources. Pietersen et al. (2017) report 86 species of amphibians for Zambia, while AmphibiaWeb (2018) reports 87 species, and a search in the Amphibian Species of the World 6.0 database (ASW; Frost 2018) returns 104 species. This disparity between databases is possibly due to the fact that the ASW includes non-confirmed occurrences. An example is the caecilian *Boulengerula*, which is expected to occur in Zambia based on its known distribution but is as yet unreported there.

Herein I present a checklist of species collected during a six-week herpetological survey in western Zambia.

Materials and Methods

Study site and sampling

The survey was carried out in April and May 2014 encompassing protected as well as non-protected areas of western Zambia (Fig. 1, Table 1). Figure 2 shows the different vegetation types surveyed, comprising *miombo* woodlands (dominated by *Brachystegia* spp.), dry evergreen forests (dominated by *Cryptosepalum* sp.), riverine forests (*mushito*) and grassy wetlands (*dambo*).

During the whole survey period there were only four

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Herpetological survey of western Zambia

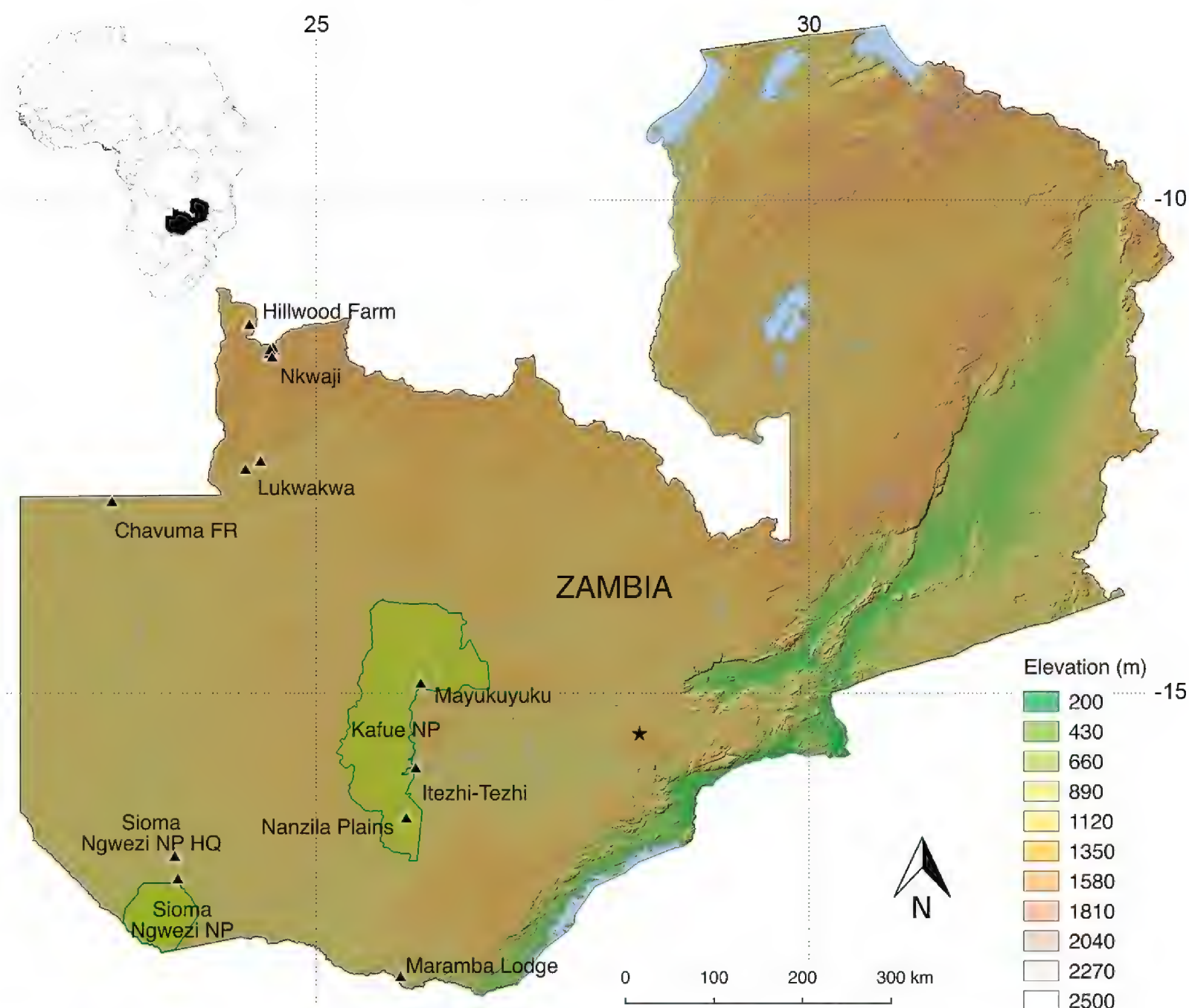


Fig. 1. Map of Zambia showing sites surveyed for herpetofauna. The star indicates the capital (Lusaka).

days of rain and average temperatures were 30 °C during the day and 15 °C at night. The main sampling methods were acoustic and visual encounter surveys (diurnal and nocturnal). Entomologists participating in the expedition opportunistically collected some specimens with the use of sweep nets and small pitfall traps (500 ml cups). All specimens collected were euthanized with 20% benzocaine (applied on the skin or in the mouth). Samples of thigh muscles were taken and stored in absolute ethanol before the specimens were fixed in 10%

formalin and transferred to 70% industrial methylated spirit for long-term storage. All specimens are deposited in the herpetological collection of the Natural History Museum in London, United Kingdom (see Appendix 1).

Species identification

Identification keys for Amphibia (Channing 2001; Poynton and Broadley 1985a) and Reptilia (Branch 1998; Broadley 1971; Broadley et al. 2003) were used to

Table 1. Localities in Zambia surveyed during this study. Protected areas are indicated by shaded green. NP: National Park; HQ: Headquarters.

Locality	District	Longitude	Latitude	Elevation (m)
Chavuma FR	Chavuma	-13.07006	22.92880	1070
Hillwood Farm	Ikelenge	-11.26316	24.32782	1400
Itezhi-Tezhi, Kafue NP	Itezhi-Tezhi	-15.77340	26.01151	1040
Lukwakwa	Kabompo	-12.66084	24.43697	1150
Livingstone, Maramba Lodge	Livingstone	-17.89120	25.85821	900
Mayukuyuku, Kafue NP	Mumbwa	-14.91533	26.06311	1010
Nanzila Plains, Kafue NP	Itezhi-Tezhi	-16.28138	25.91676	1030
Ngonye Falls Camp	Shangombo	-16.66139	23.57280	930
Nkwaji	Mwinilunga	-11.56567	24.52605	1300
Sioma Ngwezi NP	Shangombo	-16.89873	23.59847	1010
Sioma Ngwezi NP (HQ)	Shangombo	-16.66953	23.56743	1000

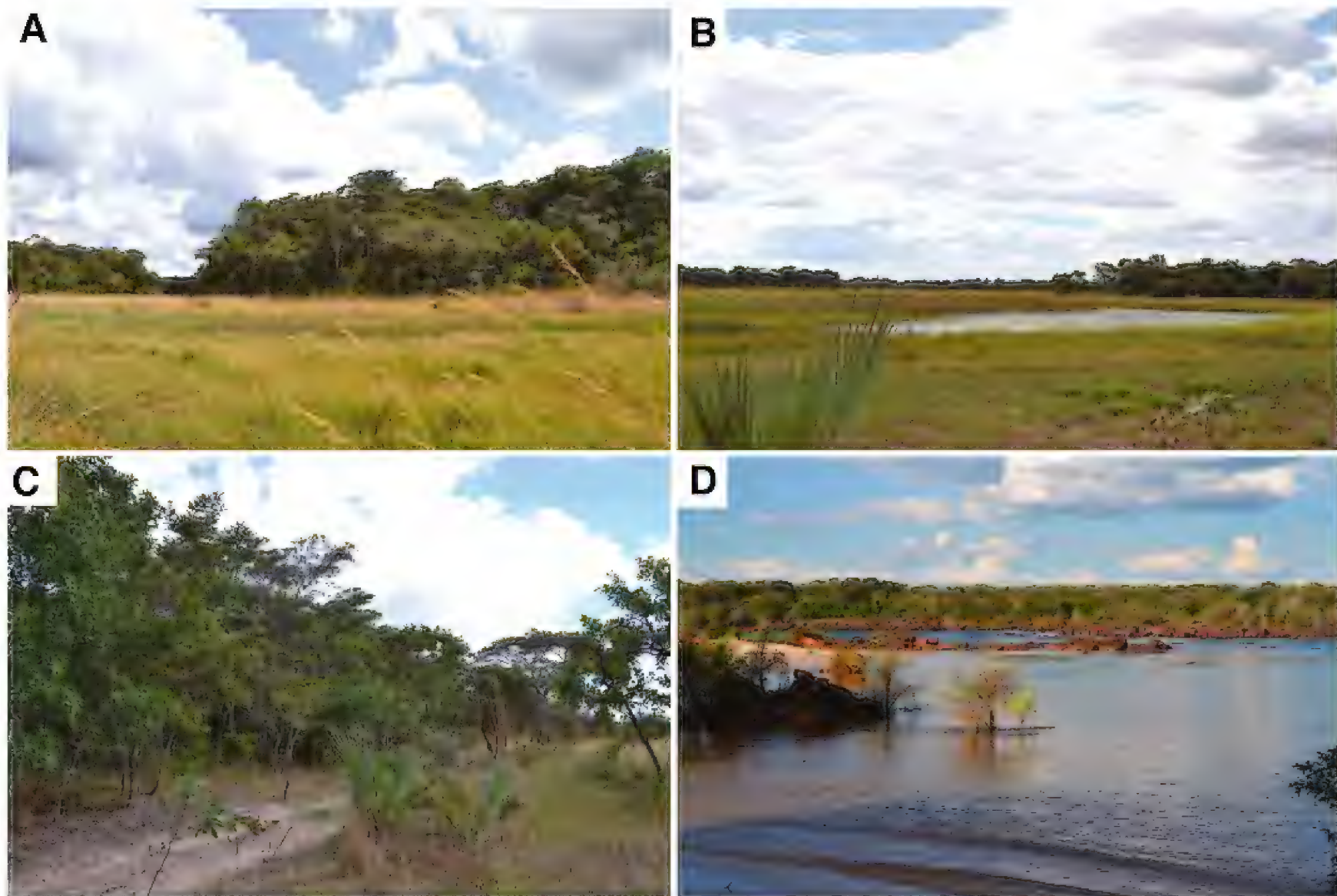


Fig. 2. Habitats surveyed in western Zambia. (A) *Dambo* and *Cryptosepalum* dry forest in Lukwakwa, (B) *Dambo* in Nanzila Plains, (C) *Miombo* woodland in Nanzila Plains, (D) Margin of the Zambezi River at Ngonye Falls.

assist with the identification of specimens. Some species identifications presented here are tentative because some groups have complex and difficult taxonomies (e.g., *Hyperolius*, *Hemisus*, *Phrynobatrachus*, and *Ptychadena*). Most samples were barcoded to help species identification (see details below). Despite its known limitations (e.g., Deichmann et al. 2017; Hebert and Gregory 2005; Meier et al. 2006), DNA barcoding is generally, and sometimes very, helpful. The Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990) was used to search the GenBank repository and identify the closest matches for each sample. As there are not many 16S rRNA sequences of Zambian reptiles and amphibians openly available for comparison, percentage of sequence similarity presented here should be interpreted with caution and while taking the possibility of geographic isolation or isolation by distance into account. Private databases were also used for sequence comparisons (D. Portik and B. Zimkus). Snakes were identified primarily using morphological characters.

Genetic analysis

Given the large amount of 16S rRNA sequence data available in GenBank for African amphibians and reptiles, this gene was selected for DNA barcoding. Total genomic DNA was extracted using a Qiagen DNeasy kit (Venlo, Netherlands) following the manufacturer's protocol for purification of total DNA from animal tissues. A fragment (ca. 500 bp) of the 16S rRNA

mitochondrial gene was amplified using the primers 16S H3062 (CCGGTTTGAAGTCAGATCA) and 16SB FROG (CGCCTGTTACCAAAAACAT) [modified from Palumbi et al. 1991]. Polymerase chain reaction (PCR) was performed using Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Life Sciences) for 35 cycles of 1 minute with annealing temperature at 51 °C. Single strand sequencing reactions and electrophoresis were carried out by the molecular lab team at the Natural History Museum in London, United Kingdom. All sequences generated are available in GenBank under accession numbers MK464267–MK464483.

DNA sequences were trimmed in Geneious v.7 (Kearse et al. 2012) with a maximum of low-quality bases of 20. Uncorrected pairwise distances (p-distances) of the 16S sequences were calculated for some groups in PAUP* (Swofford 2001). For *Phrynobatrachus*, a maximum likelihood (ML) analysis with non-parametric bootstrapping was carried out with RAxML v.8.2 (Stamatakis 2014). The alignment was generated in Geneious using the Auto algorithm of MAFFT v.7 (Katoh et al. 2002), inspected visually and poorly aligned regions were eliminated using the GBlocks Server v.091b (Castresana 2000). Evolutionary models were evaluated using Automated Model Selection (using a Neighbor Joining tree) in PAUP*. The best fitting model (GTR + GAMMA) was selected according to the Akaike Information Criteria (AIC). *Tomopterna marmorata* was used for rooting.

Results

A total of 40 species of amphibians (anurans) belonging to nine families and 13 genera, and 20 species of reptiles from nine families and 17 genera (14 lizards, five snakes, one tortoise) were recorded during this survey (Appendix 1). Among the localities surveyed, Hillwood Farm had the highest species diversity (n=23), followed by Nkwaji (n=15). Different from all other areas surveyed, which are characterized by a combination of *miombo* woodland, *dambo* and/or dry forest, both localities mentioned above have riverine or swamp forest, locally known as *mushitos* (Fig. 2).

Species accounts

All collected specimens and their respective vouchers are listed below. Voucher numbers in bold refer to specimens identified solely based on morphology (i.e., no tissue sample available).

Amphibia

Order Anura

Arthroleptidae

Arthroleptis stenodactylus Pfeffer, 1893

Shovel-footed Squeaker

Material. LUKWAKWA: BMNH 2018.5826, BMNH 2018.5827 (Fig. 3A), BMNH 2018.5828–29; NKWAJI: BMNH 2018.5830. **Comments:** Found in leaf litter in *Cryptosepalum* forest, in *dambo* and at edges of *mushito*. *Arthroleptis stenodactylus* as currently understood is widely distributed from Angola to Tanzania, and from Kenya to South Africa. This taxon clearly represents a species complex, possibly two ecologically distinct forms (see comments in Pickersgill 2007). All specimens listed above have white venters without any dark markings, large inner metatarsal tubercles and a dark line on each side running from the snout over the tympanum to the shoulder. Sequence similarity with *A. stenodactylus* from Malawi is 98% (GenBank accession numbers FJ51098–99).

Arthroleptis xenochirus Boulenger, 1905

Plain Squeaker

Material. LUKWAKWA: BMNH 2018.5811 (Fig. 3B), BMNH 2018.5812–13; HILLWOOD FARM: BMNH 2018.5814–20; NKWAJI: BMNH 2018.5821–25. **Comments:** Specimens were found in leaf litter in *Cryptosepalum* and *mushito*. All specimens have a very small tympanum and relatively large inner metatarsal tubercle (when compared to *A. xenodactyloides*). The closest match on GenBank (94%) is to *A. xenodactyloides* from Malawi (FJ151103). There is no sequence of *A. xenochirus* available for comparison.

Bufonidae

Schismaderma carens (Smith, 1848)

Red Toad

Material. CHAVUMA FR: BMNH 2018.5729; ITEZHI-TEZHI: BMNH 2018.5724–28 (Fig. 3C). **Comments:** Specimens were found in *miombo* woodland. This species is widely distributed in Zambia. The BLAST result show 100% sequence similarity with *S. carens* from South Africa (KF665176, AF220913).

Sclerophrys gutturalis (Power, 1927)

Guttural Toad

Material. CHAVUMA FR: **BMNH 2018.5703**; LIVINGSTONE: **BMNH 2018.5702**; LUKWAKWA: BMNH 2018.5705–06, **BMNH 2018.5707**; MAYUKUYUKU: BMNH 2018.5701 (Fig. 3D); NKWAJI: BMNH 2018.5704. **Comments:** This species is found in *miombo* and *Cryptosepalum* forest. These specimens lack the typical red infusions on their thighs, although this could be due to preservation. Specimen identification was confirmed using DNA barcoding (100% sequence similarity with AF220876, from Botswana).

Sclerophrys lemairii (Boulenger, 1901)

Yellow Swamp Toad

Material. HILLWOOD FARM: BMNH 2018.5723; LUKWAKWA: BMNH 2018.5715–22 (Fig. 3E). **Comments:** One male was found in a pond at night and eight individuals were found in *dambo* near the *Cryptosepalum* forest. About six males were calling during the day and two couples were observed in amplexus. The species exhibits dynamic sexual dichromatism, where males undergo a temporary color change (from dark green to bright yellow), depending on the breeding period; females are reddish, especially the parotid glands (Bittencourt-Silva 2014; Conradie and Bills 2017).

Sclerophrys pusilla (Mertens, 1937)

Southern Flat-backed Toad

Material. ITEZHI-TEZHI: BMNH 2018.5709–10; MAYUKUYUKU: BMNH 2018.5708 (Fig. 3F); NKWAJI: BMNH 2018.5711–12. **Comments:** Specimens identification were confirmed using DNA barcoding (100% sequence similarity) and non-morphometric morphological characters following Poynton et al. (2016). Specimens were found in *miombo* woodland.

Hemisotidae

Hemisus cf. *guineensis* Cope, 1865

Guinea Snout-burrower

Material. HILLWOOD FARM: BMNH 2018.5801 (juvenile); LUKWAKWA: BMNH 2018.5800 (Fig. 3G);



Fig. 3. Amphibians of western Zambia. (A) *Arthroleptis stenodactylus* (BMNH 2018.5827), (B) *Arthroleptis xenochirus* (BMNH 2018.5811), (C) *Schismaderma carens* (BMNH 2018.5728), (D) *Sclerophrys gutturalis* (BMNH 2018.5701), (E) *Sclerophrys lemairii*, (F) *Sclerophrys pusilla* (BMNH 2018.5708), (G) *Hemisus* cf. *guineensis* (BMNH 2018.5800), (H) *Hemisus* cf. *guineensis* (BMNH 2018.5799), (I) *Hemisus marmoratus* (BMNH 2018.5713), (J) *Hemisus marmoratus* (BMNH 2018.5714), (K) *Hyperolius dartevillei* (BMNH 2018.5681), (L) *Hyperolius major* (BMNH 2018.5675), (M) *Hyperolius marginatus* (BMNH 2018.5667), (N) *Hyperolius nasicus* (BMNH 2018.5666), (O) *Hyperolius parallelus* (BMNH 2018.5689), (P) *Hyperolius parallelus* (BMNH 2018.5697), (Q) *Hyperolius quinquevittatus*, (R) *Kassina senegalensis*.

SIOMA NGWEZI NP: BMNH 2018.5799 (juvenile; Fig. 3H). **Comments:** One juvenile (BMNH 2018.5801) was found dead in a pitfall trap set for dung beetles near *mushito*. One large individual (snout-vent length 42.2 mm) from Lukwakwa was found buried in sandy soil under a log between *Cryptosepalum* forest and *dambo*. The specimen from Sioma Ngwezi is a juvenile. The BLAST search shows 95% sequence similarity with *H. guineensis* from the Republic of the Congo (KY080117–19). According to Channing and Broadley (2002), *Hemissus barotseensis*, which is endemic to western Zambia, differs from *H. guineensis* and *H. marmoratus* in body proportions. The presence of a bright yellow vertebral stripe and small yellow spots on the back agree with the description of *H. barotseensis* and it is possible that these specimens are that species. If confirmed, this would represent an extension both north and south from its current known range.

Hemissus marmoratus (Peters, 1854)

Marbled Snout-burrower

Material. MAYUKUYUKU: BMNH 2018.5713 (Fig. 3I); SIOMA NGWEZI NP: BMNH 2018.5714 (Fig. 3J). **Comments:** Juvenile individuals found at night in sandy soil in *miombo* woodland. *Hemissus marmoratus* is widely distributed in sub-Saharan Africa, excluding rainforest, and it mainly inhabits savannahs but can also be found in gallery forests. The BLAST search shows the closest match on GenBank (94%) is *H. marmoratus* (AY531831). Table 2 shows the p-distance for both species of *Hemissus* presented here and highlights a limitation of the use of this measure for species delimitation.

Hyperoliidae

Hyperolius dartevelliei Laurent, 1943

Dartevelle's Reed Frog

Material. CHAVUMA FR: BMNH 2018.5681 (Fig. 3K); HILLWOOD FARM: BMNH 2018.5683–84; LUKWAKWA: BMNH 2018.5682. **Comments:** Specimens found in *miombo* woodland and edge of *mushito* basking on vegetation during the day. According to Channing et al. (2013) the snout profile of *H. dartevelliei* is truncated instead of shark-like or rounded, but none of the specimens listed above have truncated snouts. However, the BLAST results show 98–99% sequence similarity with samples of *H. dartevelliei* from Ikelenge, north-western Zambia (JQ863650, JQ863653, JQ863673, JQ863676–78, JQ863704, JQ863708, JQ863718, JQ863750, JQ863753–54, JQ863756–59, KY080197, KY080199).

Hyperolius kachalolae Schiøtz, 1975

Kachalola Reed Frog

Material. HILLWOOD FARM: BMNH 2018.5676, BMNH 2018.5677–80. **Comments:** Juvenile specimens collected during the day on vegetation near a stream in

mushito forest. In life, the overall coloration was green with a faint canthal and dorsolateral line, consisting of small spots. The green color faded after preservation, although the line is still visible. This agrees with the description provided by Schiøtz (1975). The sequenced individual shows 98% similarity with *H. kachalolae* from northern Zambia (D. Portik, pers. comm.).

Hyperolius major Laurent, 1954

Material. HILLWOOD FARM: BMNH 2018.5675 (Fig. 3L). **Comments:** One male found on top of a leaf (1.5 m from the ground) calling at night. Schiøtz (1999) reports this as a savannah species from north-western Zambia and eastern Democratic Republic of the Congo (DRC), however, this specimen was found in a forest patch (*mushito*). The closest matches from DNA barcoding (97%) are *H. kuligae* and *H. langi* (D. Portik, pers. comm.). Schiøtz (1999) states that these species found in west and central Africa are very similar in morphology and dorsal pattern, and may be conspecific. In contrast, Kohler et al. (2005) treat *H. kuligae* as a western and *H. langi* as an eastern Central African form. However, the color pattern (especially the post orbital marking) differs substantially from Laurent's description of the type material of *H. langi*, whereas this specimen agrees with the morphological description of *H. major* provided by Schiøtz (1999). There is no DNA sequence of *H. major* available for comparison.

Hyperolius marginatus Peters, 1854

Margined Reed Frog

Material. LUKWAKWA: BMNH 2018.5667 (male; Fig. 3M); NANZILA PLAINS BMNH 2018.5668, BMNH 2018.5674 (juveniles). **Comments:** Specimens found near ponds in *miombo* woodland. There is 100% sequence similarity with *H. marginatus* from Zambia (D. Portik, pers. comm.).

Hyperolius nasicus Laurent, 1943

Pointed Long Reed Frog

Material. NANZILA PLAINS BMNH 2018.5666 (Fig. 3N). **Comments:** This single specimen was collected during the day while resting on vegetation ca. 1 m above the ground in *dambo*. The specimen fits the morphological description of the species provided by Channing et al. (2013): when viewed in profile the snout has a shark-like tip, and the first, third, and fifth toes have one phalanx free (or nearly free) of webbing (see Fig. 4), distinguishing it from all the other species. The closest match on GenBank (98–99% sequence similarity) is *Hyperolius inyangae* (JQ863674, JQ863683–84), which is only known from the Eastern Highlands of Zimbabwe and Malawi (Channing et al. 2013). Although Channing et al. (2013) provide accession numbers for the genetic material of *H. nasicus*, no sequences could be found on GenBank under this species name. This could be due to sequence mislabelling and the accession numbers

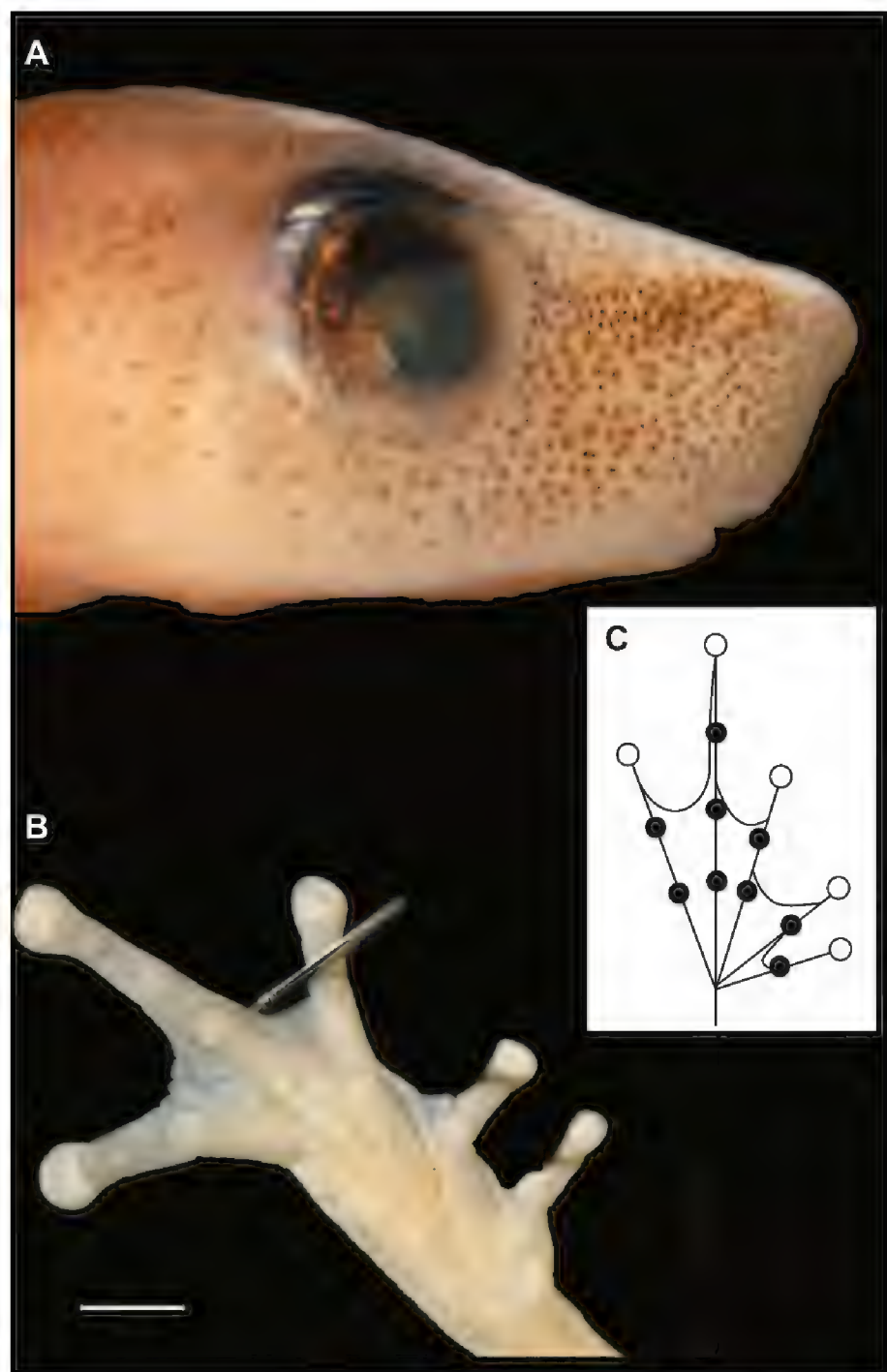


Fig. 4. Details of head profile and webbing of *Hyperolius nasicus* (BMNH 2018.5666). **(A)** Profile of head, **(B)** webbing of right foot, and **(C)** schematic representation of webbing. Scale bar represents 1 mm.

associated with *H. inyangae* may actually be from *H. nasicus*.

Hyperolius parallelus Günther, 1858

Angolan Reed Frog

Material. HILLWOOD FARM: BMNH 2018.5687–88, BMNH 2018.5689 (Fig. 3O), BMNH 2018.5690–94, **BMNH 2018.5695**; NKWAJI: BMNH 2018.5696, BMNH 2018.5697 (Fig. 3P), BMNH 2018.5698–5700.

Comments: Specimens found near ponds in *miombo* woodland. These specimens show a color variation similar to the *alborufus* group (see Schiøtz 1999). *Hyperolius parallelus* is a taxonomically complex group due to its considerable color polymorphism. According to the BLAST search, the closest match (98% sequence similarity with JQ513623, JQ513626, and JQ513625) is *H. angolensis* from Angola (see Conradie et al. 2012; Frost 2018).

Hyperolius quinquevittatus Bocage, 1866

Five-striped Reed Frog

Material. NKWAJI: **BMNH 2018.5685–86** (Fig. 3Q). **Comments:** Juveniles collected during the day while resting on vegetation in *mushito*. BLAST results show 99% sequence similarity with *H. quinquevittatus* from Ikelenge, north-western Zambia (GenBank accession number JQ863752).

Kassina senegalensis (Duméril and Bibron, 1841)

Bubbling Kassina

Material. CHAVUMA FR: BMNH 2018.5810; HILLWOOD FARM: BMNH 2018.5802–03 (Fig. 3R); NKWAJI: BMNH 2018.5804; SIOMA NGWEZI NP: BMNH 2018.5805–09. **Comments:** Specimens found in

Table 2. Uncorrected pairwise distances (p-distances) for a fragment of the 16S rRNA gene for *Hemisis* spp. Distances between conspecific populations are inside boxes.

Taxon ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 <i>Hemisis marmoratus</i> AY326070	-																
2 <i>Hemisis marmoratus</i> DQ283430	0.00	-															
3 <i>Hemisis marmoratus</i> AY531831	0.04	0.04	-														
4 <i>Hemisis marmoratus</i> KY176997	0.04	0.04	0.00	-													
5 <i>Hemisis marmoratus</i> AY948749	0.00	0.00	0.05	0.05	-												
6 <i>Hemisis marmoratus</i> KX492610	0.11	0.11	0.13	0.12	0.11	-											
7 <i>Hemisis marmoratus</i> KM509138	0.12	0.12	0.15	0.15	0.12	0.07	-										
8 <i>Hemisis marmoratus</i> KY176998	0.00	0.00	0.04	0.05	0.01	0.11	0.12	-									
9 <i>Hemisis marmoratus</i> BMNH 2018.5713	0.07	0.07	0.07	0.07	0.07	0.14	0.14	0.07	-								
10 <i>Hemisis marmoratus</i> BMNH 2018.5714	0.06	0.06	0.07	0.07	0.06	0.14	0.15	0.06	0.08	-							
11 <i>Hemisis guineensis</i> KY080117	0.12	0.12	0.13	0.13	0.13	0.14	0.15	0.13	0.14	0.15	-						
12 <i>Hemisis guineensis</i> KY080118	0.13	0.13	0.14	0.13	0.13	0.15	0.15	0.13	0.14	0.15	0.00	-					
13 <i>Hemisis guineensis</i> KY080119	0.12	0.12	0.13	0.13	0.13	0.14	0.15	0.13	0.14	0.15	0.00	0.00	-				
14 <i>Hemisis guineensis</i> KY080120	0.12	0.12	0.13	0.13	0.13	0.14	0.15	0.13	0.14	0.15	0.00	0.00	0.00	-			
15 <i>Hemisis cf guineensis</i> BMNH 2018.5799	0.13	0.13	0.15	0.15	0.14	0.14	0.15	0.14	0.15	0.16	0.05	0.05	0.05	0.05	-		
16 <i>Hemisis cf guineensis</i> BMNH 2018.5800	0.13	0.13	0.15	0.14	0.14	0.14	0.15	0.14	0.15	0.16	0.05	0.05	0.05	0.05	0.00	-	
17 <i>Hemisis cf guineensis</i> BMNH 2018.5801	0.13	0.13	0.14	0.14	0.13	0.14	0.15	0.13	0.16	0.16	0.05	0.05	0.05	0.05	0.02	0.01	-



line runs along the tibia-fibula and thigh (parallel to the band) and joins a vertebral line above the vent (see Fig. 6A). According to Du Preez and Carruthers (2017), the presence of the latter feature distinguishes *P. parvulus* from *P. mababiensis*. However, this character is present on both species and therefore cannot be used to separate them (see Poynton and Broadley 1985a. Pietersen et al. (2017) report *P. parvulus* for Ngonye Falls, approximately 25 km from Sioma Ngwesi NP, but unfortunately there is no voucher specimen. Poynton and Broadley (1985a) and Marques et al. (2018) provide discussions of the literature on the identifications of *P. mababiensis* and *P. parvulus*. The barcode is very inconclusive given that the closest hits on GenBank (92–95%) include samples of an unidentified species of *Phrynobatrachus* from Gabon (KP247505), one from the Republic of the Congo (KY080354), and *P. keniensis* (JX564885) and *P. scheffleri* (FJ889479), both from Kenya. Poynton and Broadley (1985a) suggest *P. parvulus* tends to be associated more with upland and forest conditions than

Table 3. Uncorrected pairwise distances (p-distances) for the 16S rRNA gene for *Phrynobatrachus* spp. Distances between conspecific populations are inside boxes. Dotted-line box indicates *P. natalensis* group.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 <i>Phrynobatrachus</i> cf. <i>parvulus</i> BMNH 2018.5873	-													
2 <i>Phrynobatrachus</i> cf. <i>parvulus</i> BMNH 2018.5874	0.01	-												
3 <i>Phrynobatrachus</i> cf. <i>parvulus</i> BMNH 2018.5880	0.04	0.05	-											
4 <i>Phrynobatrachus mababiensis</i> FJ889461	0.11	0.12	0.12	-										
5 <i>Phrynobatrachus mababiensis</i> BMNH 2018.5831	0.11	0.11	0.12	0.00	-									
6 <i>Phrynobatrachus mababiensis</i> BMNH 2018.5832	0.11	0.11	0.12	0.00	0.00	-								
7 <i>Phrynobatrachus natalensis</i> DQ019605	0.14	0.15	0.14	0.14	0.13	0.13	-							
8 <i>Phrynobatrachus natalensis</i> BMNH 2018.5855	0.14	0.15	0.14	0.14	0.13	0.13	0.02							
9 <i>Phrynobatrachus</i> sp. 1 BMNH 2018.5838	0.16	0.17	0.17	0.17	0.16	0.16	0.07	0.07	-					
10 <i>Phrynobatrachus</i> sp. 1 BMNH 2018.5839	0.16	0.17	0.17	0.17	0.16	0.16	0.07	0.07	0.00	-				
11 <i>Phrynobatrachus</i> sp. 3 BMNH 2018.5856	0.15	0.16	0.15	0.15	0.14	0.14	0.08	0.08	0.09	0.09	-			
12 <i>Phrynobatrachus</i> sp. 3 BMNH 2018.5858	0.15	0.16	0.15	0.16	0.15	0.15	0.08	0.09	0.09	0.09	0.01	-		
13 <i>Phrynobatrachus</i> sp. 2 BMNH 2018.5864	0.17	0.17	0.18	0.17	0.16	0.16	0.06	0.07	0.06	0.05	0.08	0.08	-	
14 <i>Phrynobatrachus</i> sp. 2 BMNH 2018.5865	0.17	0.17	0.18	0.17	0.16	0.16	0.06	0.07	0.06	0.05	0.08	0.08	0.00	-

P. mababiensis. The localities where these specimens were found are all upland and either inside or near forest, therefore I refer them to *P. cf. parvulus*.

Phrynobatrachus mababiensis FitzSimons, 1932

Dwarf Puddle Frog

Material. MAYUKUYUKU: BMNH 2018.5831–32, **BMNH 2018.5881**; NANZILA PLAINS: BMNH 2018.5833; SIOMA NGWEZI NP: BMNH 2018.5834–**35**. **Comments:** All specimens are juveniles and were found in *dambo* both during the day and at night. According to Poynton and Broadley (1985a), it is not easy to distinguish *P. mababiensis* from *P. parvulus* based on external morphology, but they suggest that some characters usually serve to separate them (i.e., labial and subtympanic markings, and shape of tarsal tubercle). The usual well-marked black and white barring on the upper and lower jaws is rather faint on these specimens. Zimkus and Schick (2010) suggest that there is cryptic diversity within the *P. mababiensis* group. The closest match on GenBank is *P. mababiensis* (FJ889461; 99% sequence similarity) from eastern Zambia, which belongs to a population that is sister to the clade containing *P. ukingensis* and *P. ungujae* (see Zimkus and Schick 2010).

Phrynobatrachus natalensis (Smith, 1849)

Snoring Puddle Frog

Material. NGONYE FALLS: BMNH 2018.5855. **Comments:** The overall external morphology resembles *P. natalensis*. The BLAST search shows 98% sequence similarity to *P. natalensis* from Mtunzini, South Africa (DQ347303), which is near Durban, the type locality of *P. natalensis* (see Table 3 and Fig. 5).

Phrynobatrachus sp. 1

Material. ITEZHI-TEZHI: BMNH 2018.5848–50; LIVINGSTONE: BMNH 2018.5853–54;

MAYUKUYUKU: BMNH 2018.5837–38 (Fig. 6B), BMNH 2018.5839–42 (Fig. 6C), BMNH 2018.5843, **BMNH 2018.5844–47**; NANZILA PLAINS: BMNH 2018.5851–52. **Comments:** Specimens were found in *dambo*s. One juvenile was found on a beach of the Zambezi River at Ngonye Falls. The closest match on GenBank (99% sequence similarity) is *P. natalensis* DQ283414 from Tanzania (see Fig. 5). Zimkus and Schick (2010) suggest that there are two species of *P. natalensis* in East Africa, and these Zambian populations are more similar to the central and southern populations corresponding to Haplotype group B. It further corresponds to Zimkus et al. (2010) *P. natalensis* Clade E. See further comments in Discussion.

Phrynobatrachus sp. 2

Material. NGONYE FALLS: BMNH 2018.5836; SIOMA NGWEZI NP: BMNH 2018.5864–65. **Comments:** Morphologically, these specimens resemble *P. natalensis* in terms of size, toe webbing and overall color pattern. However, they present silver/white spots around the vent (and ventral part of the thigh in **BMNH 2018.5836**). The closest match on GenBank is *P. natalensis* from Tanzania (95%; DQ283414). Although their range overlaps with the Southern African geographic zone (populations A and B) in Zimkus et al. (2010), these populations form a southern Zambian clade, which is a sister group of the eastern and western African clades (see Fig. 5). These findings allude to further cryptic diversity in the group.

Phrynobatrachus sp. 3

Material. HILLWOOD FARM: **BMNH 2018.5866–67** (Fig. 6D); NKWAJI: BMNH 2018.5856–**57**, BMNH 2018.5858–60, **BMNH 2018.5861** (Fig. 6E), **BMNH 2018.5862–63**; **BMNH 2018.5868–69** (Fig. 6F), **BMNH 2018.5870–72**. **Comments:** All specimens

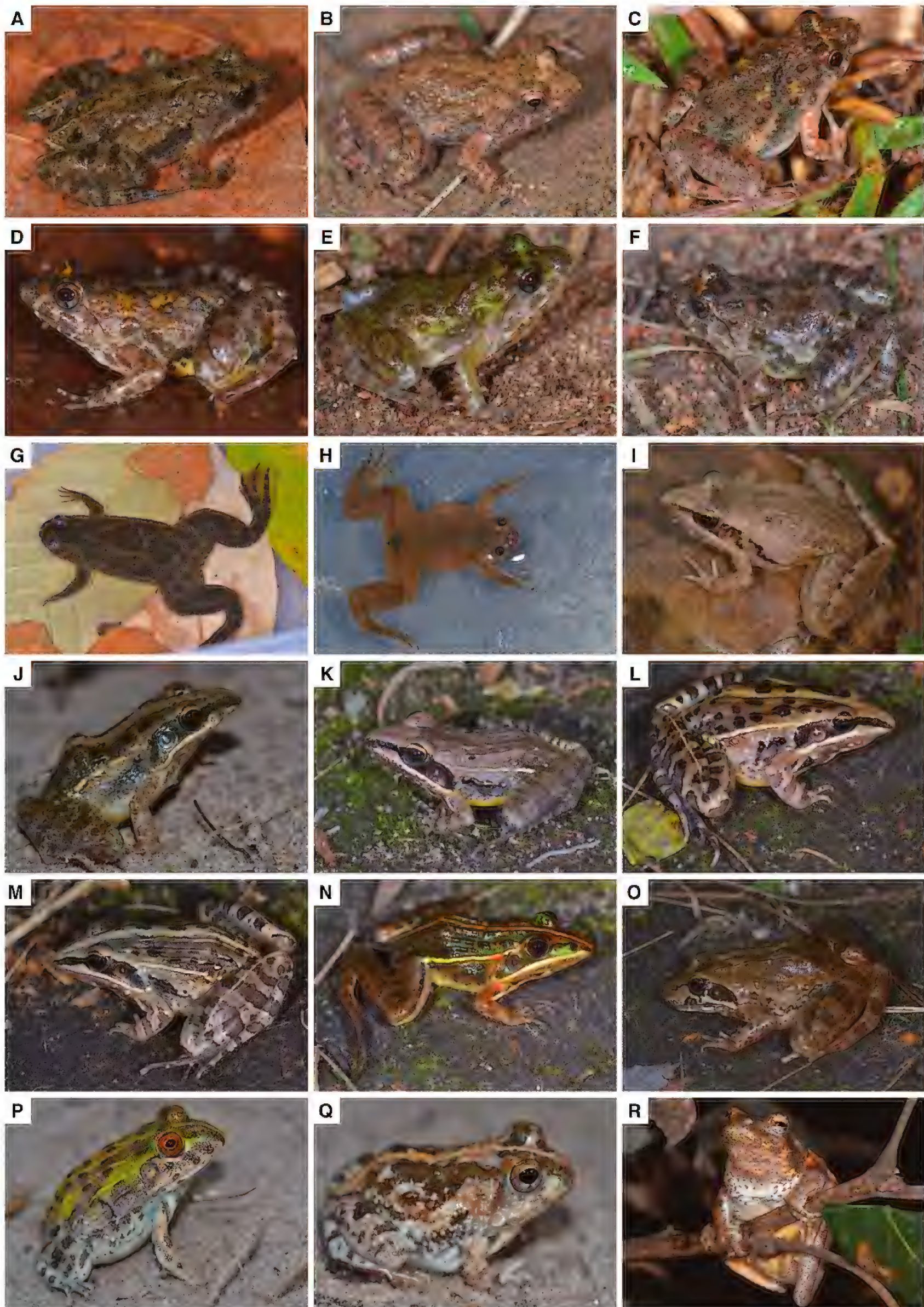


Fig. 6. Amphibians of western Zambia. (A) *Phrynobatrachus* cf. *parvulus* (BMNH 2018.5889), (B) *Phrynobatrachus natalensis* (BMNH 2018.5838), (C) *Phrynobatrachus natalensis* (BMNH 2018.5842), (D) *Phrynobatrachus* sp. 1 (BMNH 2018.5867), (E) *Phrynobatrachus* sp. 1 (BMNH 2018.5861), (F) *Phrynobatrachus* sp. 1 (BMNH 2018.5869), (G) *Xenopus poweri* (BMNH 2018.5659), (H) *Xenopus pygmaeus*, (I) *Ptychadena anchietae* (BMNH 2018.5730), (J) *Ptychadena* cf. *mossambica* (BMNH 2018.5759), (K) *Ptychadena porosissima* (BMNH 2018.5766), (L) *Ptychadena porosissima* (BMNH 2018.5769), (M) *Ptychadena porosissima* (BMNH 2018.5770), (N) *Ptychadena taenioscelis* (BMNH 2018.5785), (O) *Amietia chapini* (BMNH 2018.5664), (P) *Pyxicephalus* cf. *adspersus* (BMNH 2018.5791), (Q) *Tomopterna* sp. (BMNH 2018.5797), (R) *Chiromantis xerampelina* (BMNH 2018.5798).

are morphologically similar to *P. natalensis*, however the BLAST search shows sequence similarity with *P. natalensis* from South Africa (GenBank accession number DQ347303) varying between 90–91% (see Fig. 5). These populations form a northern Zambian clade, sister to southern, western, and eastern African clades. Further investigation is needed to resolve the taxonomical status of this group.

Pipidae

Xenopus poweri Hewitt, 1927

Power's Clawed Frog

Material. HILLWOOD FARM: BMNH 2018.5654–58; NKWAJI: BMNH 2018.5659 (Fig. 6G). **Comments:** Specimens collected from a pond in *miombo* area. The BLAST search shows 99% sequence similarity with *X. poweri* from Ikelenge, north-western Zambia (GenBank accession number KP345253). Furman et al. (2015) removed this name from the synonymy of *X. petersii* and reassigned the West African populations of *X. laevis* to this species.

Xenopus pygmaeus Loumont, 1986

Bouchia Clawed Frog

Material. HILLWOOD FARM: BMNH 2018.5651–52 (Fig. 6H), BMNH 2018.5653. **Comments:** Species identification was based on their relatively small size and the presence of a fourth claw. Specimens were found in a small pool formed in a car track next to a riverine forest (*mushito*). Recently, Wagner et al. (2013) presented the first record of *Xenopus pygmaeus* for Zambia, representing a significant range extension (about 1,300 km). This species belongs to the *fraseri* subgroup and it was previously known to have its southernmost distribution in the northern part of the DRC. The DNA barcode corroborates the morphological identification (99% sequence similarity with KF738291).

Ptychadenidae

Ptychadena anchietae (Bocage, 1868)

Anchieta's Ridged Frog

Material. ITEZHI-TEZHI: BMNH 2018.5735–36; MAYUKUYUKU: BMNH 2018.5730–31 (Fig. 6I), BMNH 2018.5732–34. **Comments:** Specimens collected near water in *miombo* woodland. The closest match (99%) is *P. anchietae* (AY517610) from Tanzania.

Ptychadena grandisonae Laurent, 1954

Grandison's Ridged Frog

Material. NKWAJI: BMNH 2018.5737–43. **Comments:** Two juveniles and four males found in a pond in *miombo*. This series fits the description for *P. grandisonae* in Poynton and Broadley (1985a). The results of the BLAST search show that the closest match (98%) is

Ptychadena sp. (GenBank accession number KF178892) from Gabon.

Ptychadena cf. *guibei*

Material. MAYUKUYUKU: BMNH 2018.5764; SIOMA NGWEZI NP: BMNH 2018.5765. **Comments:** Specimens identified following the key in Poynton and Broadley (1985a). Foot length of BMNH 2018.5765 is slightly less than half the body length and thus, according to the key, this specimen would be *mossambica* or *cotti* (i.e. *schillukorum*). The BLAST result shows 96% sequence similarity with *P. porosissima* (KY177058) from Kenya. There is no sequence of *P. guibei* available for comparison.

Ptychadena mapacha Channing, 1993

Mapach Ridged Frog

Material. MAYUKUYUKU: BMNH 2018.5772 (male). **Comments:** Specimen collected near water in *miombo* woodland. Following Poynton and Broadley (1985a), this specimen should be assigned to *P. cotti*, now a synonym of *P. schillukorum*. However, this specimen also fits the description of *Ptychadena mapacha* (not included in the key), except for the white spots on the posterior face of the tibia and a thin tibial line that are present in the holotype (CAS 160535). The closest match (89%) is *P. porosissima* (GenBank accession number KY177058) from Kenya, and the sequence similarity with *P. schillukorum* (KY177060) is 82%. There is no sequence of *P. mapacha* available for comparison. Although *P. schillukorum* is listed in the AmphibiaWeb database as occurring in Zambia, there is no reference to the literature confirming this claim. Pietersen et al. (2017) provided an unconfirmed record of *P. mapacha* for Sioma Ngwesi. This record represents the northernmost record of this species.

Ptychadena cf. *mossambica* (Peters, 1854)

Mozambique Ridged Frog

Material. ITEZHI-TEZHI: BMNH 2018.5753, BMNH 2018.5754–57; MAYUKUYUKU: BMNH 2018.5763; SIOMA NGWEZI NP: BMNH 2018.5758–59 (Fig. 6J), BMNH 2018.5760, BMNH 2018.5761. **Comments:** The key in Poynton and Broadley (1985a) points to *P. mossambica*, except for the skin folds that are not continuous in these specimens. The authors note that *P. mossambica* shows an east-west cline in size and degree of webbing, where material from western Zambia tends to be smaller (maximum SVL 28.9 mm) than the series from Mozambique (maximum SVL 52.5 mm). The average SVL of this series is 34 mm. Most specimens have a pair (sometimes two) of large dark blotches on the scapular region. The closest match on GenBank (93–94%) is *Ptychadena* cf. *mossambica* from coastal Tanzania (KY177057). These specimens may be referable to *P. mapacha* Channing, 1993, for which there is no available sequence data. *Ptychadena mapacha*

has recently been recorded in Ngonye Falls, south-west Zambia by Pietersen et al. (2017), confirming Channing's (2001) prediction about its distribution.

Ptychadena nilotica (Seetzen, 1855)

Nile Grass Frog

Material. LIVINGSTONE: BMNH 2018.5781; NANZILA PLAINS: BMNH 2018.5773–76, BMNH 2018.5777–80. **Comments:** Specimens collected at night near water in *miombo* woodland. Sequence similarity with *P. nilotica* is 98% with KF027211 and 99% with KX836515 from the DRC. For further discussion about this species see Dehling and Sinsch (2013) and Zimkus et al. (2016).

Ptychadena obscura (Schmidt and Inger, 1959)

Material. HILLWOOD FARM: BMNH 2018.5766–67 (Fig. 6K), BMNH 2018.5768. **Comments:** These small specimens (SVL ranges from 21.8 to 22.8 mm) fit the description of *P. obscura* in Poynton and Broadley (1985a). The specimens have a pair of dark marks on the scapular region. The results of the BLAST search show higher similarity (97–98%) to *P. broadleyi* (GenBank accession number MH300600–02). This is an unexpected finding considering that *P. broadleyi* is only known to occur in the Mulanje Mountain and the Zomba Plateau, in Malawi. These specimens from Zambia have a light triangle on the snout distinguishing them from *P. broadleyi*. Hence, the barcoding results should be interpreted with caution.

Ptychadena oxyrhynchus (Smith, 1849)

Sharp-nosed Grass Frog

Material. HILLWOOD FARM: BMNH 2018.5783; NANZILA PLAINS: BMNH 2018.5782. **Comments:** Specimens collected near water in *miombo* woodland. Sequence similarity with *P. oxyrhynchus* from Kwambonambi, South Africa (GenBank accession number AF215403) is 99%.

Ptychadena porosissima (Steindachner, 1867)

Three-striped Grass Frog

Material. HILLWOOD FARM: BMNH 2018.5769 (Fig. 6L), BMNH 2018.5770 (Fig. 6M), BMNH 2018.5771. **Comments:** This species, common in *miombo* woodland near water bodies, was present in large numbers at Hillwood Farm. Sequence similarity with *P. porosissima* (GenBank accession number KF027212) from Rwanda is 98%.

Ptychadena cf. *taenioscelis* Laurent, 1954

Stripe-legged Grass Frog

Material. HILLWOOD FARM: BMNH 2018.5784–86 (Fig. 6N). **Comments:** Adult specimens found in pond. These specimens have been identified using the key in Poynton and Broadley (1985a). The closest match on GenBank (95%) is *P. taenioscelis* from the Republic of the

Congo (GenBank accession number KY080397). Perret (1979) assigned records of *taenioscelis* from west and central Africa to *pumilio* Boulenger. There seems to be some confusion in the literature regarding the taxonomy of these species, and a review of the group is needed.

Ptychadena upembae (Schmidt and Inger, 1959)

Upemba Ridged Frog

Material. HILLWOOD FARM: BMNH 2018.5750–52 (last number is a juvenile); NKWAJI: BMNH 2018.5744–46 (juveniles), BMNH 2018.5747–49 (last number is a juvenile); SIOMA NGWEZI NP: **BMNH 2018.5762**. **Comments:** Following the key in Poynton and Broadley (1985a), this series should be assigned to *Ptychadena upembae*. The BLAST search shows that the closest match (96%) is *Ptychadena* aff. *porosissima* (GenBank accession number DQ525940) from Tanzania, but it is important to note that there is no sequence of *P. upembae* available for comparison.

Pyxicephalidae

Amietia chapini (Noble, 1924)

Chapin's River Frog

Material. HILLWOOD FARM: BMNH 2018.5660–61, **BMNH 2018.5662–63**, **BMNH 2018.5664** (Fig. 6O), BMNH 2018.5665. **Comments:** Specimens found near streams in *miombo* woodland. The result of the BLAST search shows that *A. chapini* is the closest match to the specimens collected at Hillwood Farm (sequence similarity with *A. chapini* of 96–98%). All specimens have long legs (tibiofibula ~0.6 of snout-vent length), as noted by Noble (1924). I note that the specimens described as *A. chapini* by Channing et al. (2016) differ from the specimens listed above in coloration – the latter being darker. If these specimens are confirmed to be *A. chapini*, this will be the first record of this species for Zambia, but their presence in Zambia is not surprising considering the proximity (ca. 380 km) between the currently known populations from southern DRC (Kundelungu National Park) and Hillwood Farm.

Pyxicephalus cf. *adspersus* Tschudi, 1838

Giant Bullfrog

Material. SIOMA NGWEZI NP: BMNH 2018.5787–91 (Fig. 6P). **Comments:** All individuals collected are juveniles. Hence, identification is tentative and based on the geographic range of the species. The BLAST shows 95% sequence similarity to *Pyxicephalus* cf. *adspersus* (DQ347304) and *P. edulis* (DQ022366).

Tomopterna marmorata (Peters, 1854)

Marbled Sand Frog

Material. LIVINGSTONE: BMNH 2018.5792. **Comments:** Juvenile found in a small pond at night. Skin of dorsum and venter is smooth; venter is pale

Table 4. Uncorrected pairwise distances (p-distances) for the 16S rRNA gene for *Tomopterna* spp. Distances between conspecific populations are inside boxes.

	1	2	3	4	5	6	7	8	9	10	11
1 <i>Tomopterna</i> sp. "Shankara" AY255095	-										
2 <i>Tomopterna</i> sp. BMNH 2018.5793	0.01	-									
3 <i>Tomopterna</i> sp. BMNH 2018.5794	0.01	0.00	-								
4 <i>Tomopterna</i> sp. BMNH 2018.5795	0.01	0.00	0.00	-							
5 <i>Tomopterna</i> sp. BMNH 2018.5796	0.01	0.00	0.00	0.00	-						
6 <i>Tomopterna</i> sp. BMNH 2018.5797	0.01	0.00	0.00	0.00	0.00	-					
7 <i>Tomopterna delalandii</i> DQ283403	0.06	0.06	0.05	0.05	0.05	0.05	-				
8 <i>Tomopterna damarensis</i> KX869909	0.07	0.06	0.06	0.06	0.06	0.06	0.02	-			
9 <i>Tomopterna cryptotis</i> JX564898	0.06	0.06	0.06	0.06	0.06	0.06	0.03	0.02	-		
10 <i>Tomopterna marmorata</i> BMNH 2018.5792	0.08	0.08	0.07	0.07	0.07	0.07	0.06	0.05	0.06	-	
11 <i>Tomopterna marmorata</i> AY255084	0.08	0.08	0.07	0.07	0.07	0.07	0.06	0.05	0.06	0.00	-

with a few dark markings under the throat; tympanum indistinguishable; undivided sub-articular tubercle on first finger. The closest match on GenBank (100%) is *Tomopterna marmorata* (AY255084) from Zambia.

Tomopterna sp.

Material. SIOMA NGWEZI NP: BMNH 2018.5793–97 (Fig. 6Q). **Comments:** All individuals are juveniles and were found at night close to a light-trap set to collect beetles. The main morphological characters of these specimens are: dorsal and ventral skin smooth; immaculate venter; presence of a ridge below tympanum; tympanum indistinguishable; and undivided sub-articular tubercle on first finger. The closest match on GenBank (99%) is *Tomopterna* sp. “Shankara” (AY255095), an undescribed species from Namibia (Dawood et al. 2002). Table 4 shows the p-distances among the closest matches from GenBank.

Rhacophoridae

Chiromantis xerampelina Peters, 1854

African Grey Treefrog

Material. MAYUKUYUKU: BMNH 2018.5798 (Fig. 6R). **Comments:** This is a widespread species found in *miombo* woodland. One adult individual found at night on a tree near the campsite.

Reptilia

Order Squamata

Agamidae

Agama armata Peters, 1855

Northern Ground Agama

Material. NGONYE FALLS CAMP: BMNH 2018.2751 (Fig. 7A). **Comments:** One juvenile found basking on a log by the Zambezi River. Sequence similarity is

98% with *A. armata* ZFMK 84990 (GenBank accession number GU128447).

Chamaeleonidae

Chamaeleo dilepis Leach, 1819

Flap-necked Chameleon

Material. CHAVUMA FR: BMNH 2018.2755 (Fig. 7B), BMNH 2018.2756. **Comments:** Specimens were found in *miombo* woodland on shrubs above 1 m. There are currently seven subspecies in this group and based on their distributions, these specimens represent *C. dilepis quilensis* (Uetz et al. 2018). Sequence similarity is 99% with *Chamaeleo dilepis* from Matema, Tanzania (GenBank accession number AY927272).

Gekkonidae

Hemidactylus mabouia (Moreau de Jonnès, 1818)

Common Tropical House Gecko

Material. ITEZHI-TEZHI: BMNH 2018.2742, **BMNH 2018.2740**; NANZILA PLAINS: BMNH 2018.2741. **Comments:** Common species found in a variety of habitats, including heavily degraded ones, though not found in forests. The closest matches from GenBank are *Hemidactylus mercatorius* (AY863034) and *H. mabouia* (AY863038), both showing 94% sequence similarity.

Lygodactylus chobiensis Fitzsimons, 1932

Chobe Dwarf Gecko

Material. ITEZHI-TEZHI: BMNH 2018.2743 (female; Fig. 7C), **BMNH 2018.2744** (male) **Comments:** Specimens found on tree-trunks in *miombo*. Identification follows the key provided by Broadley (1971). The rostral is excluded from the nostril and the male has dark forward-directed chevron marks on the throat. The female is yellow and white underneath (Fig. 7D). The closest match from GenBank (95% sequence similarity)



Fig. 7. Reptiles of western Zambia. (A) *Agama armata* (BMNH 2018.2751), (B) *Chamaeleo dilepis* (BMNH 2018.2755), (C)–(D) *Lygodactylus chobiensis* (BMNH 2018.2743), (E) *Pachydactylus punctatus*, (F) *Ichnotropis capensis* (BMNH 2018.2750), (G) *Meroles squamulosus* (BMNH 2018.2753), (H) *Trachylepis* cf. *albopunctata* (BMNH 2018.2765), (I) *Trachylepis varia* (BMNH 2018.2769), (J) *Typhlacontias rohani* (BMNH 2018.2761), (K) *Crotaphopeltis hotamboeia* (BMNH 2018.2776), (L) *Philothamnus hoplogaster* (BMNH 2018.2775), (M) *Rhamnophis aethiopissa ituriensis* (BMNH 2018.2772), (N) *Thelotornis kirtlandii* (BMNH 2018.2760), (O) *Atractaspis congica* (BMNH 2018.2274), (P)–(R) *Kinixys spekii*.

is *Lygodactylus chobiensis* from Zimbabwe (GenBank accession number GU593456).

Lygodactylus angolensis Bocage, 1896

Angola Dwarf Gecko

Material. NKWAJI: BMNH 2018.2767 (male), BMNH 2018.2766 (female). **Comments:** Specimens were identified following the key provided by Broadley (1971). In both specimens, the mental has a pair of lateral clefts, resulting from fusion with a large postmental. The male has nine preanal pores, which distinguish it from *L. bradfieldi*. The closest match from GenBank (90% sequence similarity) is *Lygodactylus* sp. from East Africa (GenBank accession numbers GU593448–50).

Pachydactylus punctatus Peters, 1854

Speckled Thick-toed Gecko

Material. ITEZHI-TEZHI: BMNH 2018.2757, BMNH 2018.2758 (Fig. 7E), **BMNH 2018.2759.** **Comments:** Specimens found at night in dry leaf litter in *miombo* woodland. All specimens have the dorsum covered with sub-uniform granules. The closest matches from GenBank (93% sequence similarity) are *Pachydactylus punctatus* (AF449120) and *P. scherzi* (AY123379). As the latter is only known from Namibia (Bauer and Branch 1995), I assign these specimens to *P. punctatus*.

Gerrhosauridae

Gerrhosaurus bulsi Laurent, 1954

Laurent's Plated Lizard

Material. HILLWOOD FARM: BMNH 2018.2754. **Comments:** One juvenile collected by the farm scouts in dry *miombo*. The BLAST search shows the closest match (98%) as *Gerrhosaurus bulsi* from Angola (KF717381). Broadley (1971, 1991) referred the population of *Gerrhosaurus* from Ikelenge (north-western Zambia) to *multilineatus* but this was later contested by Haagner et al. (2000). Bates et al. (2013) consider *G. bulsi* a valid species and discuss the taxonomic problems regarding *G. multilineatus*.

Lacertidae

Ichnotropis capensis (Smith, 1838)

Cape Rough-scaled Lizard

Material. CHAVUMA FR: BMNH 2018.2746; LUKWAKWA: **BMNH 2018.2749**, BMNH 2018.2747; NANZILA PLAINS: BMNH 2018.2750, **BMNH 2018.2748** (Fig. 7F); SIOMA NGWEZI NP: BMNH 2018.2745. **Comments:** All specimens are juveniles and were found in *miombo* woodland. Sequence similarity is 99% with *I. capensis* from Katima Mulilo, Namibia (GenBank accession number JX962898).

Meroles squamulosus (Peters, 1854)

Savanna Lizard

Material. NANZILA PLAINS: BMNH 2018.2753 (Fig. 7G); SIOMA NGWEZI NP: BMNH 2018.2752.

Comments: Two adult males found in *miombo*. Sequence similarity is 96% with *Meroles (Ichnotropis) squamulosus* from Laela, Tanzania (GenBank accession number JX962897).

Scincidae

Panaspis cf. *wahlbergi* (Smith, 1849)

Snake-eyed Skink

Material. CHAVUMA FR: BMNH 2018.2738, **BMNH 2018.2739.** **Comments:** Specimens found during the day in leaf litter. The closest match from GenBank (98%) is *Panaspis* sp. (KU236726), from Katanga, DRC. Medina et al. (2016) provide a molecular phylogeny of this genus, which suggests that there is cryptic diversity within *P. wahlbergi*.

Trachylepis cf. *albopunctata* (Bocage, 1867)

Angolan Variable Skink

Material. ITEZHI-TEZHI: BMNH 2018.2762; MAYUKUYUKU: BMNH 2018.2765 (Fig. 7H), **BMNH 2018.2763.** **Comments:** Specimens found during the day. The BLAST search shows 99–100% sequence similarity with sequences from *T. varia* clade B (accession numbers MG605651–59), which was recently assigned to *Trachylepis* cf. *albopunctata* by Marques et al. (2018).

Trachylepis damarana (Peters, 1870)

Damara Skink

Material. SIOMA NGWEZI NP (HQ): BMNH 2018.2764. **Comments:** Morphologically similar to *T. varia* group. However, both its distribution and sequence similarity (99%) match *T. damarana* (see Weinell and Bauer 2018).

Trachylepis wahlbergii (Peters, 1869)

Wahlberg's Striped Skink

Material. ITEZHI-TEZHI: BMNH 2018.2769 (Fig. 7I); LUKWAKWA: **BMNH 2018.2768**, BMNH 2018.2770; NKWAJI: BMNH 2018.2771. **Comments:** Specimens found during the day on rocks (Itezhi-Tezhi), *dambo* (Lukwakwa), and inside a tree trunk at Nkwaji. According to Broadley (2000) these specimens fall in the distribution range of *T. wahlbergii*. The most similar sequence from GenBank is *T. wahlbergii* from Zambia (99%, accession number DQ234810).

Typhlacontias rohani Angel, 1923

Rohan's Blind Dart Skink

Material. SIOMA NGWEZI NP (HQ): BMNH 2018.2761 (Fig. 7J). **Comments:** One specimen was found buried in sand and collected by Errol Pietersen. The closest match on GenBank (90%) is *T. punctatissimus* (DQ316889). There is no sequence of *T. rohani* available

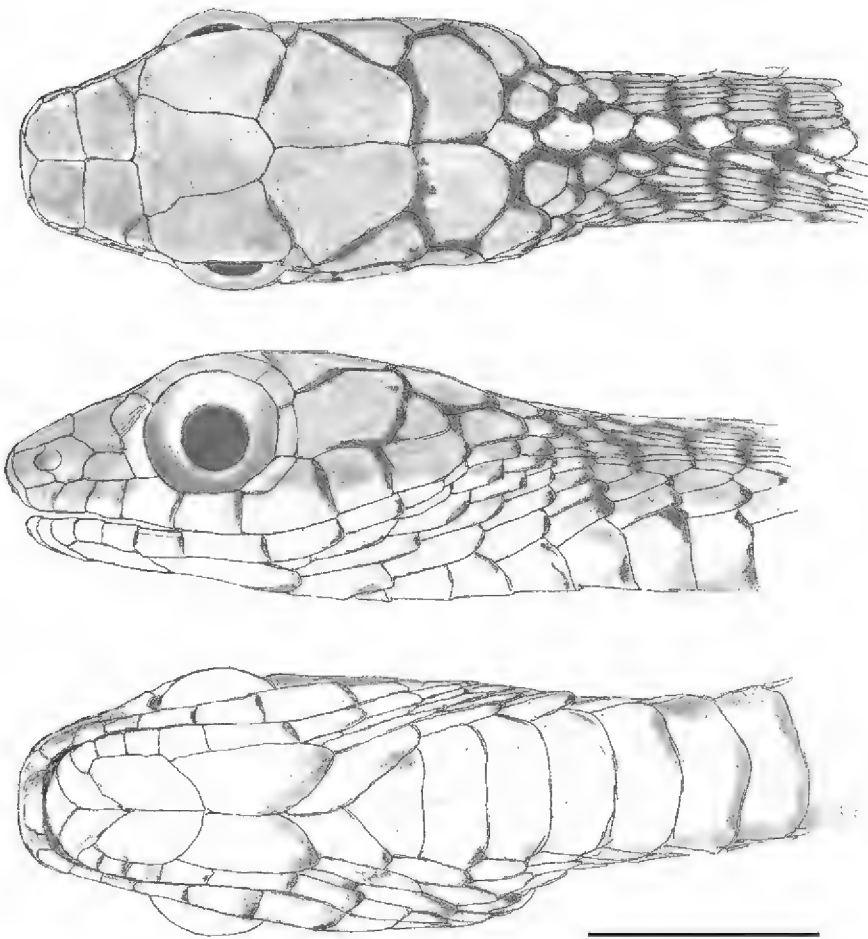


Fig. 8. Drawings of *Rhamnophis aethiopissa ituriensis* (BMNH 2018.2772). Head and anterior of body in dorsal, left lateral, and ventral views. Scale bar 10 mm. Drawings by Ed Wade.

for comparison.

Colubridae

Crotaphopeltis hotamboeia (Laurenti, 1768)

White-lipped Herald Snake

Material. ITEZHI-TEZHI: BMNH 2018.2776 (Fig. 7K); HILLWOOD FARM: BMNH 2018.2773; SIOMA NGWEZI NP: BMNH 2018.2777. **Comments:** All individuals collected are juveniles and were found at night in *miombo* woodland near rocks. The most similar sequence on GenBank is *C. hotamboeia* from Malawi (99%, accession number AY611816).

Philothamnus hoplogaster (Günther, 1863)

Green Water Snake

Material. NANZILA PLAINS: BMNH 2018.2775 (Fig. 7L). **Comments:** Specimen found while eating tadpoles and juveniles of *Kassina* sp. in a temporary pond during the day. This species is similar to other *Philothamnus* but usually smaller (Marais 2004). The closest match on GenBank (99%) is *P. hoplogaster* from Mozambique (accession number FJ913484).

Rhamnophis aethiopissa ituriensis (Schmidt 1923)

Large-eyed Green Tree Snake

Material. HILLWOOD FARM: BMNH 2018.2772 (Fig. 7M; Fig. 8). **Comments:** Specimen found in leaf litter of riverine forest during the day. Broadley (1991) provided the first record of this species for Zambia. Based on distribution, this form represents the subspecies *R. a. ituriensis* from Niapu in the DRC (see Eimermacher 2012).

Thelotornis kirtlandii (Hallowell, 1844)

Forest Vine Snake

Material. HILLWOOD FARM: BMNH 2018.2760 (Fig. 7N). **Comments:** One juvenile was found at night resting on green vegetation in riverine forest (*mushito*). Species was identified using the key in Broadley (2001) and the following characters were observed: top of the head, including temporal region, is uniform green; rostral and nasals are strongly recurved onto top of snout; supralabials are white with small green spots.

Lamprophiidae

Atractaspis congica Peters, 1877

Congo Stiletto Snake

Material. HILLWOOD FARM: BMNH 2018.2274 (Fig. 7O). **Comments:** One relatively large specimen found in moist leaf litter inside a patch of *mushito* at night. Broadley and Blaylock (2013): 232 expanded the description of *A. bibronii* to accommodate the condition of 19 midbody scales of *A. congica* (19–21 scales at midbody). This specimen exhibits erratic counts of 19+17, the latter count predominating after the 84th ventral.

Order Testudines

Testudinidae

Kinixys spekii Gray, 1863

Speke's Hinge-back Tortoise

Comments: One specimen (Figs. 5P–R) was found in *mushito* at Nkwaji. The carapace was hinged between the 7th and 8th marginals. The specimen was photographed and released.

Discussion

This is a non-comprehensive list of the herpetofauna of western Zambia. The survey was conducted shortly after the general breeding season for amphibians and reptiles in this part of the world, and consequently most specimens collected are juveniles. For the same reason, most species were not active, which made the search for them more challenging. However, some species of amphibians were active during the survey. *Phrynobatrachus* were heard calling during the day and at night, and at least ten males of *Sclerophrys lemairii* were calling during the day in Lukwakwa (see Bittencourt-Silva 2014). The presence of juveniles of *Ichnotropis capensis* and adults of *Meroles squamulosus* in sympatry is explained by their staggered life cycles (see Broadley 1967, 1979).

DNA barcoding is an important tool for identifying candidate species. However, there are a number of caveats. For instance, for amphibians, Vences et al. (2005a) propose a tentative 16S rRNA threshold at 5% for interspecific sequence divergence but also

highlight the broad overlap of intra- and interspecific divergence values (see Vences et al. 2005b, p. 1,865) that complicates the establishment of threshold values. Using DNA barcoding alone can potentially lead to simplistic diagnoses of putative species. Another issue with DNA barcoding relates to the taxonomic accuracy of public DNA databases (e.g., GenBank, BOLD, EMBL). Misidentified sequences are not uncommon (Bridge et al. 2003; Vilgalys 2003), which reinforces the importance of vouchering all sequences deposited. Table 2 shows that the intra-specific p-distances within *H. marmoratus* are considerably large in some cases. This could be partly due to geographic distances, given that the specimens are from Ghana, Guinea-Bissau, Kenya, and Tanzania. A taxonomic review of this group is clearly necessary. Nonetheless, genetic data may be crucial in cases where species are genetically different but morphologically largely conserved. An example is the mongrel frogs from Mozambique and Malawi, which have ca. 5% interspecific divergences but are in general phenotypically indistinguishable (Conradie et al. 2018).

Some of the taxa reported here could not be assigned to currently recognized species based on DNA barcoding and/or external morphology. For instance, based on p-distances, some of the *Phrynobatrachus* specimens represent putative new species (Table 3). Zimkus and Schick (2010) suggest that there are at least two species currently identified as *Phrynobatrachus natalensis* in East Africa, and another two clades are reported from western and southern Africa (Zimkus et al. 2010). The phylogeny presented here indicates that there may be more species of this group in western Zambia (see Fig. 5). Species identified here as *P. cf. parvulus* may be a new species. These results corroborate the conclusions of Zimkus and Schick (2010) and Zimkus et al. (2010) that a taxonomic review of the genus *Phrynobatrachus* is needed. Similarly, the *Tomopterna* population found in Sioma Ngwezi NP could represent an undescribed species (Table 4) previously reported from Namibia (see Dawood et al. 2002). These taxa deserve further investigations by specialists. It is often the case that original species descriptions lack diagnostic details, including illustration of characters, and/or type material may be lost or in poor condition, all of which can contribute to inconclusive or even incorrect species identification. Additional datasets (e.g., bioacoustics, ecology) often provide important information and can solve some of these taxonomic conundrums.

The genus *Ptychadena* currently comprises 56 species, some (possibly many) representing species complexes (e.g., Zimkus et al. 2016). Nineteen species have been reported in Zambia (see genus account in Frost 2018), eleven of which were recorded during this survey. The lack of an updated key to the *Ptychadena* of Zambia makes the species identification process challenging. Similarly, barcoding is not helpful when there are no reference sequences available. A search of

GenBank for 16S sequences of *Ptychadena* shows that 41% of currently recognized species are not represented, and 22% of the sequences available are either pending confirmation or identified only to genus. The taxonomy of this group is clearly in need of attention.

Except for a few areas—on the extreme north-west of the country and along the Zambezi river—most of Zambia remains poorly studied. Recently, Channing and Willems (2018) described a new species of *Ptychadena* from the northern part of the country, and a new cryptic species of *Polemon* (Squamata: Lamprophiidae) described from the DRC and Uganda is likely to occur in Zambia (Portillo et al. 2019). The list of species provided here adds new points to the map of the Zambian herpetofauna.

The herpetofauna of Zambia is mostly contained in the Zambezian biogeographical core, with only the south-western region forming part of the South African core (*sensu* Linder et al. 2012). Not surprisingly, many species found during this survey also occur in the DRC (e.g., Channing et al. 2016), Angola (Conradie et al. 2016) and Namibia (Dawood et al. 2002), including *Amietia chapini*, recorded here for the first time from Zambia. Four species of amphibians that Pietersen et al. (2017) expected to occur near Ngonye Falls are now confirmed to occur at Sioma Ngwezi NP (*Kassina senegalensis*, *Phrynobatrachus mababiensis*, *Ptychadena porosissima*, and *Pyxicephalus adspersus*). The still very incomplete knowledge of the Zambian herpetofauna remains the main obstacle to our understanding of its biogeography and the conservation statuses of its constituent species.

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Literature Cited

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.
- AmphibiaWeb. 2018. Available: <https://amphibiaweb>.

- org. [Accessed: 17 July 2018].
- Bates MF, Tolley KA, Edwards S, Davids Z, Da Silva JM, Branch WR. 2013. A molecular phylogeny of the African plated lizards, genus *Gerrhosaurus* Wiegmann, 1828 (Squamata: Gerrhosauridae), with the description of two new genera. *Zootaxa* 3750: 465–493.
- Bauer AM, Branch WR. 1995. Geographic variation in western populations of the *Pachydactylus punctatus* complex (Reptilia: Gekkonidae). *Tropical Zoology* 8: 69–84.
- Bittencourt-Silva GB. 2014. Notes on the reproductive behaviour of *Amietophrynus lemairii* (Boulenger, 1901) (Anura: Bufonidae). *Herpetology Notes* 7: 611–614.
- Branch WR. 1998. *Field Guide to the Snakes and other Reptiles of Southern Africa*. Struik, Cape Town, South Africa. 399 p.
- Bridge PD, Roberts PJ, Spooner BM, Panchal G. 2003. On the unreliability of published DNA sequences. *New Phytologist* 160: 43–48.
- Broadley DG. 1967. The life cycles of two sympatric species of *Ichnotropis* (Sauria: Lacertidae). *African Zoology* 3: 1–2.
- Broadley DG. 1971. *The Reptiles and Amphibians of Zambia*. The Puku, Number 6. Kenya Litho, Nairobi, Kenya. 143 p.
- Broadley DG. 1979. A field study of two sympatric annual lizards (genus *Ichnotropis*) in Rhodesia. *African Zoology* 14: 133–138.
- Broadley DG. 1991. The herpetofauna of northern Mwinilunga District, northwestern Zambia. *Arnoldia Zimbabwe* 9: 519–538.
- Broadley DG. 2000. A review of the genus *Mabuya* in southeastern Africa (Sauria: Scincidae). *African Journal of Herpetology* 49: 87–110.
- Broadley DG. 2001. A review of the genus *Thelotornis* A. Smith in eastern Africa, with the description of a new species from the Usambara Mountains (Serpentes: Colubridae: Dispholidini). *African Journal of Herpetology* 50(2): 53–70.
- Broadley DG, Blaylock R. 2013. *Snakes of Zimbabwe and Botswana*. Edition Chimaira, Frankfurt am Main, Germany. 387 p.
- Broadley DG, Doria C, Wigge J. 2003. *Snakes of Zambia: An Atlas and Field Guide*. Edition Chimaira, Frankfurt am Main, Germany. 280 p.
- Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540–552.
- Channing A. 2001. *Amphibians of Central and Southern Africa*. Comstock Publishing Associates, Ithaca, New York, USA. 470 p.
- Channing A, Broadley DG. 2002. A new snout-burrower from the Barotse floodplain (Anura: Hemisotidae: *Hemisus*). *Journal of Herpetology* 36: 367–372.
- Channing A, Willems F. 2018. A new grass frog with rupicolous tadpoles from northern Zambia (Anura: Ptychadenidae). *Zootaxa* 4462: 349–366.
- Channing A, Hillers A, Lötters S, Rödel M-O, Schick S, Conradie W, Rödder D, Mercurio V, Wagner P, Dehling JM. 2013. Taxonomy of the super-cryptic *Hyperolius nasutus* group of long reed frogs of Africa (Anura: Hyperoliidae), with descriptions of six new species. *Zootaxa* 3620: 301–350.
- Channing A, Dehling JM, Lötters S, Ernst R. 2016. Species boundaries and taxonomy of the African river frogs (Amphibia: Pyxicephalidae: *Amietia*). *Zootaxa* 4155: 1–76.
- Conradie W, Bills R. 2017. Wannabe Ranid: Notes on the morphology and natural history of the Lemaire's Toad (Bufonidae: *Sclerophrys lemairii*). *Salamandra* 53: 439–444.
- Conradie W, Branch WR, Measey GJ, Tolley KA. 2012. A new species of *Hyperolius* Rapp, 1842 (Anura: Hyperoliidae) from the Serra da Chela mountains, south-western Angola. *Zootaxa* 3269: 1–17.
- Conradie W, Bills R, Branch WR. 2016. The herpetofauna of the Cubango, Cuito, and lower Cuando river catchments of south-eastern Angola. *Amphibian & Reptile Conservation* 10(2) [Special Section]: 6–36 (e126).
- Conradie W, Bittencourt-Silva GB, Farooq HM, Loader SP, Menegon M, Tolley KA. 2018. New species of Mongrel Frogs (Pyxicephalidae: *Nothophryne*) for northern Mozambique inselbergs. *African Journal of Herpetology* 67: 61–85.
- Dawood A, Channing A, Bogart JP. 2002. A molecular phylogeny of the frog genus *Tomopterna* in southern Africa: examining species boundaries with mitochondrial 12S rRNA sequence data. *Molecular Phylogenetics and Evolution* 22: 407–413.
- Dehling JM, Sinsch U. 2013. Diversity of *Ptychadena* in Rwanda and taxonomic status of *P. chrysogaster* Laurent, 1954 (Amphibia, Anura, Ptychadenidae). *ZooKeys* 356: 69–102.
- Deichmann JL, Mulcahy DG, Vanthomme H, Tobi E, Wynn AH, Zimkus BM, McDiarmid RW. 2017. How many species and under what names? Using DNA barcoding and GenBank data for west Central African amphibian conservation. *PLoS ONE* 12: e0187283.
- Eimermacher TG. 2012. Phylogenetic systematics of Dispholidine colubrids (Serpentes: Colubridae). Ph.D. Dissertation, The University of Texas at Arlington, Arlington, Texas, USA. 109 p.
- Frost DR. 2018. Amphibian sSpecies of the World: an online reference. Version 6.0. American Museum of Natural History, New York, New York, USA. Available: <http://research.amnh.org/vz/herpetology/amphibia/index.php> [Accessed: 10 November 2018].
- Furman BLS, Bewick AJ, Harrison TL, Greenbaum E, Gvoždík V, Kusamba C, Evans BJ. 2015. Pan-African phylogeography of a model organism, the African

- clawed frog *Xenopus laevis*. *Molecular Ecology* 24: 909–925.
- Haagner GV, Branch WR, Haagner AJF. 2000. Notes on a collection of reptiles from Zambia and adjacent areas of the Democratic Republic of the Congo. *Annals of the Eastern Cape Museum* 1: 1–25.
- Hebert PDN, Gregory TR. 2005. The promise of DNA barcoding for taxonomy. *Systematic Biology* 54: 852–859.
- Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3,059–3,066.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1,647–1,649.
- Köhler J, Scheelke K, Schick S, Veith M, Lotters S. 2005. Contribution to the taxonomy of hyperoliid frogs (Amphibia: Anura: Hyperoliidae): advertisement calls of twelve species from East and Central Africa. *African Zoology* 40: 127–142.
- Linder HP, de Klerk HM, Born J, Burgess ND, Fjeldså J, Rahbek C. 2012. The partitioning of Africa: statistically defined biogeographical regions in sub-Saharan Africa. *Journal of Biogeography* 39: 1,189–1,205.
- Maraïs J. 2004. *A Complete Guide to the Snakes of Southern Africa*. Struik Nature, Cape Town, South Africa. 740 p.
- Marques MP, Ceríaco LMP, Blackburn DC, Bauer AM. 2018. Diversity and distribution of the amphibians and terrestrial reptiles of Angola. Atlas of historical and bibliographic records (1840–2017). *Proceedings of the California Academy of Sciences* 65(Supplement 1): 1–501.
- Medina MF, Bauer AM, Branch WR, Schmitz A, Conradie W, Nagy ZT, Hibbitts TJ, Ernst R, Portik DM, Nielsen SV. 2016. Molecular phylogeny of *Panaspis* and *Afroablepharus* skinks (Squamata: Scincidae) in the savannas of sub-Saharan Africa. *Molecular Phylogenetics and Evolution* 100: 409–423.
- Meier R, Shiyang K, Vaidya G, Ng PKL. 2006. DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology* 55: 715–728.
- Noble GK. 1924. Contributions to the herpetology of the Belgian Congo based on the collection of the American Museum Congo Expedition, 1909–1915. Part 3, Amphibia. *Bulletin of the American Museum of Natural History* 49(2): 147–347.
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991. The Simple Fool's Guide to PCR. Version 2.0. Privately published document compiled by S. Palumbi. Department of Zoology, University of Hawaii, Honolulu, Hawaii, USA.
- Perret JL. 1979. Remarques et mise au point sur quelques espèces de *Ptychadena* (Amphibia, Ranidae). *Bulletin de la Société Neuchâteloise des Sciences Naturelles* 102: 5–21.
- Phiri P. 2005. *A Checklist of Zambian Vascular Plants*. SABONET, Pretoria, South Africa. 169 p.
- Pickersgill M. 2007. *Frog Search, Results of Expeditions to Southern and Eastern Africa*. Frankfurt Contributions to Natural History. Editions Chimaira, Frankfurt am Main, Germany. 574 p.
- Pietersen DW, Pietersen EW, Conradie W. 2017. Preliminary herpetological survey of Ngonye Falls and surrounding regions in south-western Zambia. *Amphibian & Reptile Conservation* 11(1) [Special Section]: 24–43 (e148).
- Portillo F, Branch WR, Tilbury CR, Nagy ZT, Hughes DF, Kusamba C, Muninga WM, Behangana M, Greenbaum E. 2019. A cryptic new species of *Polemon* (Squamata: Lamprophiidae, Aparallactinae) from the Miombo woodlands of Central and East Africa. *Copeia* 107: 22–36.
- Poynton JC, Broadley DG. 1985a. Amphibia Zambesiaca 2. Ranidae. *Annals of the Natal Museum* 27: 115–181.
- Poynton JC, Broadley DG. 1985b. Amphibia Zambesiaca 1. Scolecomorphidae, Pipidae, Microhylidae, Hemisidae, Arthroleptidae. *Annals of the Natal Museum* 26: 503–553.
- Poynton JC, Broadley DG. 1987. Amphibia Zambesiaca 3. Rhacophoridae and Hyperoliidae. *Annals of the Natal Museum* 28: 161–229.
- Poynton JC, Broadley DG. 1988. Amphibia Zambesiaca 4. Bufonidae. *Annals of the Natal Museum* 29: 447–490.
- Poynton JC, Broadley DG. 1991. Amphibia Zambesiaca 5. Zoogeography. *Annals of the Natal Museum* 39: 221–277.
- Poynton JC, Loader SP, Conradie W, Roedel M-O, Liedtke HC. 2016. Designation and description of a neotype of *Sclerophrys maculata* (Hallowell, 1854), and reinstatement of *S. pusilla* (Mertens, 1937) (Amphibia: Anura: Bufonidae). *Zootaxa* 4098: 73–94.
- Du Preez L, Carruthers V. 2017. *Frogs of Southern Africa: A Complete Guide*. Struik Nature, Cape Town, South Africa. 519 p.
- Schiotz A. 1975. *Treefrogs of Eastern Africa*. Steenstrupia, Copenhagen, Denmark. 232 p.
- Schiotz A. 1999. *Treefrogs of Africa*. Andreas S. Brahm, Frankfurt am Main, Germany. 350 p.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1,312–1,313.
- Swofford DL. 2001. PAUP*: Phylogenetic Analysis Using Parsimony (and other methods) 4.0.b5. Sinauer Associates, Sunderland, Massachusetts, USA.
- Uetz P, Freed P, Hošek J. 2018. *The Reptile Database*.

- Available: <http://www.reptile-database.org> [Accessed: 15 February 2019].
- Vences M, Thomas M, Bonett RM, Vieites DR. 2005a. Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360: 1,859–1,868.
- Vences M, Thomas M, van der Meijden A, Chiari Y, Vieites DR. 2005b. Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology* 2: 1–12.
- Vilgalys R. 2003. Taxonomic misidentification in public DNA databases. *New Phytologist* 160: 4–5.
- Wagner P, Wilms TM, Rödder D, Schmitz A. 2013. A great leap—the first record of *Xenopus pygmaeus* (Anura: Pipidae) from south of the Congo Basin. *Salamandra* 49: 206–210.
- Weinell JL, Bauer AM. 2018. Systematics and phylogeography of the widely distributed African skink *Trachylepis varia* species complex. *Molecular Phylogenetics and Evolution* 120: 103–117.
- Zimkus BM, Schick S. 2010. Light at the end of the tunnel: insights into the molecular systematics of East African puddle frogs (Anura: Phrynobatrachidae). *Systematics and Biodiversity* 8: 39–47.
- Zimkus BM, Rödel MO, Hillers A. 2010. Complex patterns of continental speciation: Molecular phylogenetics and biogeography of sub-Saharan puddle frogs (*Phrynobatrachus*). *Molecular Phylogenetics and Evolution* 55: 883–900.
- Zimkus BM, Lawson LP, Barej M, Barratt CD, Channing A, Dash KM, Dehling JM, Du Preez L, Gehring P-S, Greenbaum E, et al. 2016. Leapfrogging into new territory: How Mascarene ridged frogs diversified across Africa and Madagascar to maintain their ecological niche. *Molecular Phylogenetics and Evolution* 106: 254–269.



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Appendix 1. List of amphibians and reptiles found in western Zambia, including species vouchers, GenBank accession numbers, and locality information. Museum acronym: BMNH – Natural History Museum, London, United Kingdom. GPS datum WGS-84.

Species	Voucher ID	Field ID	GenBank	Locality	Latitude	Longitude	Altitude
AMPHIBIA: ANURA							
ARTHROLEPTIDAE							
<i>Arthroleptis stenodactylus</i>	BMNH 2018.5826	SL 2109	MK464479	Lukwakwa	-12.66084	24.43697	1063
<i>Arthroleptis stenodactylus</i>	BMNH 2018.5827	SL 2121	MK464478	Lukwakwa	-12.66084	24.43697	1063
<i>Arthroleptis stenodactylus</i>	BMNH 2018.5828	SL 2123	MK464477	Lukwakwa	-12.66084	24.43697	1063
<i>Arthroleptis stenodactylus</i>	BMNH 2018.5829	SL 2128	MK464476	Lukwakwa	-12.66084	24.43697	1063
<i>Arthroleptis stenodactylus</i>	BMNH 2018.5830	SL 2221	MK464475	Nkwaji	-11.57728	24.53960	1291
<i>Arthroleptis xenochirus</i>	BMNH 2018.5814	SL 2145	MK464471	Hillwood Farm	-11.26316	24.32782	1356
<i>Arthroleptis xenochirus</i>	BMNH 2018.5815	SL 2146	MK464470	Hillwood Farm	-11.26316	24.32782	1356
<i>Arthroleptis xenochirus</i>	BMNH 2018.5816	SL 2147	MK464469	Hillwood Farm	-11.26316	24.32782	1356
<i>Arthroleptis xenochirus</i>	BMNH 2018.5817	SL 2148	MK464468	Hillwood Farm	-11.26316	24.32782	1356
<i>Arthroleptis xenochirus</i>	BMNH 2018.5818	SL 2152	MK464467	Hillwood Farm	-11.26690	24.31666	1308
<i>Arthroleptis xenochirus</i>	BMNH 2018.5819	SL 2153	MK464466	Hillwood Farm	-11.26690	24.31666	1308
<i>Arthroleptis xenochirus</i>	BMNH 2018.5820	SL 2246	MK464465	Hillwood Farm	-11.26690	24.31666	1308
<i>Arthroleptis xenochirus</i>	BMNH 2018.5811	SL 2122	MK464474	Lukwakwa	-12.66084	24.43697	1063
<i>Arthroleptis xenochirus</i>	BMNH 2018.5812	SL 2124	MK464473	Lukwakwa	-12.66084	24.43697	1063
<i>Arthroleptis xenochirus</i>	BMNH 2018.5813	SL 2125	MK464472	Lukwakwa	-12.66084	24.43697	1063
<i>Arthroleptis xenochirus</i>	BMNH 2018.5821	SL 2200	MK464464	Nkwaji	-11.60592	24.55448	1244
<i>Arthroleptis xenochirus</i>	BMNH 2018.5822	SL 2201	MK464463	Nkwaji	-11.60592	24.55448	1244
<i>Arthroleptis xenochirus</i>	BMNH 2018.5823	SL 2202	MK464462	Nkwaji	-11.60592	24.55448	1244
<i>Arthroleptis xenochirus</i>	BMNH 2018.5824	SL 2203	MK464461	Nkwaji	-11.60592	24.55448	1244
<i>Arthroleptis xenochirus</i>	BMNH 2018.5825	SL 2204	MK464460	Nkwaji	-11.60592	24.55448	1244
BUFONIDAE							
<i>Sclerophrys gutturalis</i>	BMNH 2018.5703	SL 2102		Chavuma FR	-13.07006	22.92880	1073
<i>Sclerophrys gutturalis</i>	BMNH 2018.5705	SL 2108	MK464294	Lukwakwa	-12.66084	24.43697	1063
<i>Sclerophrys gutturalis</i>	BMNH 2018.5706	SL 2107	MK464293	Lukwakwa	-12.74275	24.28436	1101
<i>Sclerophrys gutturalis</i>	BMNH 2018.5707	SL 2111		Lukwakwa	-12.66084	24.43697	1063
<i>Sclerophrys gutturalis</i>	BMNH 2018.5702	SL 2069	MK464296	Maramba Lodge, Livingstone	-17.89120	25.85821	900
<i>Sclerophrys gutturalis</i>	BMNH 2018.5701	SL 2025	MK464297	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Sclerophrys gutturalis</i>	BMNH 2018.5704	SL 2190	MK464295	Nkwaji	-11.60592	24.55448	1244
<i>Sclerophrys lemairii</i>	BMNH 2018.5723	SL 2245		Hillwood Farm	-11.26690	24.31666	1308
<i>Sclerophrys lemairii</i>	BMNH 2018.5715	SL 2112		Lukwakwa	-12.66084	24.43697	1063
<i>Sclerophrys lemairii</i>	BMNH 2018.5716	SL 2113		Lukwakwa	-12.66084	24.43697	1063
<i>Sclerophrys lemairii</i>	BMNH 2018.5717	SL 2114		Lukwakwa	-12.66084	24.43697	1063
<i>Sclerophrys lemairii</i>	BMNH 2018.5718	SL 2115		Lukwakwa	-12.66084	24.43697	1063
<i>Sclerophrys lemairii</i>	BMNH 2018.5719	SL 2116		Lukwakwa	-12.66084	24.43697	1063
<i>Sclerophrys lemairii</i>	BMNH 2018.5720	SL 2117		Lukwakwa	-12.66084	24.43697	1063
<i>Sclerophrys lemairii</i>	BMNH 2018.5721	SL 2118		Lukwakwa	-12.66084	24.43697	1063
<i>Sclerophrys lemairii</i>	BMNH 2018.5722	SL 2119		Lukwakwa	-12.66084	24.43697	1063
<i>Sclerophrys pusilla</i>	BMNH 2018.5709	SL 2047	MK464291	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Sclerophrys pusilla</i>	BMNH 2018.5710	SL 2053	MK464290	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Sclerophrys pusilla</i>	BMNH 2018.5708	SL 2012	MK464292	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Sclerophrys pusilla</i>	BMNH 2018.5711	SL 2189	MK464289	Nkwaji	-11.60592	24.55448	1244

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Appendix 1 (continued). List of amphibians and reptiles found in western Zambia, including species vouchers, GenBank accession numbers, and locality information. Museum acronym: BMNH – Natural History Museum, London, United Kingdom. GPS datum WGS-84.

Species	Voucher ID	Field ID	GenBank	Locality	Latitude	Longitude	Altitude
<i>Sclerophrys pusilla</i>	BMNH 2018.5712	SL 2191	MK464288	Nkwaji	-11.60592	24.55448	1244
<i>Schismaderma carens</i>	BMNH 2018.5729	SL 2105	MK464298	Chavuma FR	-13.07006	22.92880	1073
<i>Schismaderma carens</i>	BMNH 2018.5724	SL 2048	MK464303	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Schismaderma carens</i>	BMNH 2018.5725	SL 2049	MK464302	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Schismaderma carens</i>	BMNH 2018.5726	SL 2050	MK464301	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Schismaderma carens</i>	BMNH 2018.5727	SL 2051	MK464300	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Schismaderma carens</i>	BMNH 2018.5728	SL 2052	MK464299	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
HEMISOTIDAE							
<i>Hemisus</i> cf. <i>guineensis</i>	BMNH 2018.5801	SL 2252	MK464449	Hillwood Farm	-11.26690	24.31666	1308
<i>Hemisus</i> cf. <i>guineensis</i>	BMNH 2018.5800	SL 2110	MK464450	Lukwakwa	-12.66084	24.43697	1063
<i>Hemisus</i> cf. <i>guineensis</i>	BMNH 2018.5799	SL 2091	MK464451	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Hemisus marmoratus</i>	BMNH 2018.5713	SL 2018	MK464448	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Hemisus marmoratus</i>	BMNH 2018.5714	SL 2090	MK464447	Sioma Ngwezi NP	-16.89873	23.59847	1009
HYPEROLIIDAE							
<i>Hyperolius dartevelliei</i>	BMNH 2018.5681	SL 2098	MK464446	Chavuma FR	-13.07006	22.92880	1073
<i>Hyperolius dartevelliei</i>	BMNH 2018.5683	SL 2139	MK464444	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius dartevelliei</i>	BMNH 2018.5684	SL 2140	MK464443	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius dartevelliei</i>	BMNH 2018.5682	SL 2127	MK464445	Lukwakwa	-12.66084	24.43697	1063
<i>Hyperolius kachalolae</i>	BMNH 2018.5676	SL 2138	MK464442	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius kachalolae</i>	BMNH 2018.5677	SL 2149	MK464441	Hillwood Farm	-11.26316	24.32782	1356
<i>Hyperolius kachalolae</i>	BMNH 2018.5678	SL 2180	MK464440	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius kachalolae</i>	BMNH 2018.5679	SL 2243	MK464439	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius kachalolae</i>	BMNH 2018.5680	SL 2244	MK464438	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius major</i>	BMNH 2018.5675	SL 2159	MK464437	Hillwood Farm	-11.27444	24.32444	1416
<i>Hyperolius marginatus</i>	BMNH 2018.5667	SL 2126	MK464436	Lukwakwa	-12.66091	24.42943	1100
<i>Hyperolius marginatus</i>	BMNH 2018.5668	SL 2063	MK464435	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Hyperolius marginatus</i>	BMNH 2018.5674	SL 2064	MK464434	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Hyperolius nasicus</i>	BMNH 2018.5666	SL 2056	MK464433	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Hyperolius paralellus</i>	BMNH 2018.5687	SL 2133	MK464432	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius paralellus</i>	BMNH 2018.5688	SL 2136	MK464431	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius paralellus</i>	BMNH 2018.5689	SL 2137	MK464430	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius paralellus</i>	BMNH 2018.5690	SL 2141	MK464429	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius paralellus</i>	BMNH 2018.5691	SL 2142	MK464428	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius paralellus</i>	BMNH 2018.5692	SL 2150	MK464427	Hillwood Farm	-11.26316	24.32782	1356
<i>Hyperolius paralellus</i>	BMNH 2018.5693	SL 2161	MK464426	Hillwood Farm	-11.27444	24.32444	1416
<i>Hyperolius paralellus</i>	BMNH 2018.5694	SL 2179	MK464425	Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius paralellus</i>	BMNH 2018.5695	SL 2184		Hillwood Farm	-11.26690	24.31666	1308
<i>Hyperolius paralellus</i>	BMNH 2018.5696	SL 2224	MK464424	Nkwaji	-11.50420	24.56456	1386
<i>Hyperolius paralellus</i>	BMNH 2018.5697	SL 2225	MK464423	Nkwaji	-11.50420	24.56456	1386
<i>Hyperolius paralellus</i>	BMNH 2018.5698	SL 2220	MK464422	Nkwaji	-11.60592	24.55448	1244
<i>Hyperolius paralellus</i>	BMNH 2018.5699	SL 2240	MK464421	Nkwaji	-11.53906	24.55262	1336
<i>Hyperolius paralellus</i>	BMNH 2018.5700	SL 2241	MK464420	Nkwaji	-11.53906	24.55262	1336
<i>Hyperolius quinquevittatus</i>	BMNH 2018.5685	SL 2216	MK464419	Nkwaji	-11.53906	24.55262	1336

Appendix 1 (continued). List of amphibians and reptiles found in western Zambia, including species vouchers, GenBank accession numbers, and locality information. Museum acronym: BMNH – Natural History Museum, London, United Kingdom. GPS datum WGS-84.

Species	Voucher ID	Field ID	GenBank	Locality	Latitude	Longitude	Altitude
<i>Hyperolius quinquevittatus</i>	BMNH 2018.5686	SL 2217		Nkwaji	-11.53906	24.55262	1336
<i>Kassina senegalensis</i>	BMNH 2018.5810	SL 2106	MK464406	Chavuma FR	-13.07006	22.92880	1073
<i>Kassina senegalensis</i>	BMNH 2018.5802	SL 2129	MK464414	Hillwood Farm	-11.26690	24.31666	1308
<i>Kassina senegalensis</i>	BMNH 2018.5803	SL 2130	MK464413	Hillwood Farm	-11.26690	24.31666	1308
<i>Kassina senegalensis</i>	BMNH 2018.5804	SL 2223	MK464412	Nkwaji	-11.50420	24.56456	1386
<i>Kassina senegalensis</i>	BMNH 2018.5805	SL 2073	MK464411	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Kassina senegalensis</i>	BMNH 2018.5806	SL 2074	MK464410	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Kassina senegalensis</i>	BMNH 2018.5807	SL 2075	MK464409	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Kassina senegalensis</i>	BMNH 2018.5808	SL 2076	MK464408	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Kassina senegalensis</i>	BMNH 2018.5809	SL 2087	MK464407	Sioma Ngwezi NP	-16.89873	23.59847	1009
PHRYNOBATRACHIDAE							
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5873	SL 2157	MK464397	Hillwood Farm	-11.26316	24.32782	1356
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5874	SL 2162	MK464396	Hillwood Farm	-11.27444	24.32444	1416
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5875	SL 2163	MK464395	Hillwood Farm	-11.26316	24.32782	1356
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5876	SL 2164	MK464394	Hillwood Farm	-11.26316	24.32782	1356
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5877	SL 2165	MK464393	Hillwood Farm	-11.26316	24.32782	1356
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5878	SL 2166	MK464392	Hillwood Farm	-11.26316	24.32782	1356
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5889	SL 2120	MK464389	Lukwakwa	-12.66084	24.43697	1063
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5879	SL 2208	MK464391	Nkwaji	-11.56594	24.52659	1311
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5880	SL 2210	MK464390	Nkwaji	-11.56594	24.52659	1311
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5882	SL 2205		Nkwaji	-11.56594	24.52659	1311
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5883	SL 2234		Nkwaji	-11.56567	24.52605	1263
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5884	SL 2235		Nkwaji	-11.56567	24.52605	1263
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5885	SL 2236		Nkwaji	-11.56567	24.52605	1263
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5886	SL 2237		Nkwaji	-11.56567	24.52605	1263
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5887	SL 2238		Nkwaji	-11.56567	24.52605	1263
<i>Phrynobatrachus</i> cf. <i>parvulus</i>	BMNH 2018.5888	SL 2239		Nkwaji	-11.56567	24.52605	1263
<i>Phrynobatrachus mababiensis</i>	BMNH 2018.5831	SL 2010	MK464388	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus mababiensis</i>	BMNH 2018.5832	SL 2011	MK464387	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus mababiensis</i>	BMNH 2018.5881	SL 2015		Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus mababiensis</i>	BMNH 2018.5833	SL 2065	MK464386	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Phrynobatrachus mababiensis</i>	BMNH 2018.5834	SL 2080	MK464385	Sioma Ngwezi NP	-16.89873	23.59847	1009

Herpetological survey of western Zambia

Appendix 1 (continued). List of amphibians and reptiles found in western Zambia, including species vouchers, GenBank accession numbers, and locality information. Museum acronym: BMNH – Natural History Museum, London, United Kingdom. GPS datum WGS-84.

Species	Voucher ID	Field ID	GenBank	Locality	Latitude	Longitude	Altitude
<i>Phrynobatrachus mababiensis</i>	BMNH 2018.5835	SL 2081		Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5848	SL 2045	MK464377	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5849	SL 2046	MK464376	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5850	SL 2054	MK464375	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5853	SL 2066	MK464372	Maramba Lodge, Livingstone	-17.89120	25.85821	900
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5854	SL 2067	MK464371	Maramba Lodge, Livingstone	-17.89120	25.85821	900
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5837	SL 2016	MK464384	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5838	SL 2017	MK464383	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5839	SL 2026	MK464382	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5840	SL 2027	MK464381	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5841	SL 2033	MK464380	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5842	SL 2034	MK464379	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5843	SL 2037	MK464378	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5844	SL 2028		Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5845	SL 2029		Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5846	SL 2030		Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5847	SL 2031		Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5851	SL 2055	MK464374	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Phrynobatrachus natalensis</i>	BMNH 2018.5852	SL 2057	MK464373	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5866	SL 2143	MK464364	Hillwood Farm	-11.26316	24.32782	1356
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5867	SL 2144	MK464363	Hillwood Farm	-11.26316	24.32782	1356
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5856	SL 2195	MK464369	Nkwaji	-11.60592	24.55448	1244
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5857	SL 2196		Nkwaji	-11.60592	24.55448	1244
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5858	SL 2197	MK464368	Nkwaji	-11.60592	24.55448	1244
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5859	SL 2198	MK464367	Nkwaji	-11.60592	24.55448	1244
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5860	SL 2199	MK464366	Nkwaji	-11.60592	24.55448	1244
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5861	SL 2211		Nkwaji	-11.56594	24.52659	1311
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5862	SL 2219		Nkwaji	-11.56567	24.52605	1263
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5863	SL 2232	MK464365	Nkwaji	-11.56567	24.52605	1263
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5868	SL 2206	MK464362	Nkwaji	-11.56594	24.52659	1311
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5869	SL 2207	MK464361	Nkwaji	-11.56594	24.52659	1311
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5870	SL 2209	MK464360	Nkwaji	-11.56594	24.52659	1311
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5871	SL 2231	MK464359	Nkwaji	-11.56567	24.52605	1263
<i>Phrynobatrachus</i> sp.1	BMNH 2018.5872	SL 2233	MK464358	Nkwaji	-11.56567	24.52605	1263
<i>Phrynobatrachus</i> sp.2	BMNH 2018.5836	SL 2071	MK464357	Ngonye Falls Camp	-16.66139	23.57280	929
<i>Phrynobatrachus</i> sp.2	BMNH 2018.5864	SL 2095	MK464356	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Phrynobatrachus</i> sp.2	BMNH 2018.5865	SL 2096	MK464355	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Phrynobatrachus</i> sp.3	BMNH 2018.5855	SL 2072	MK464370	Ngonye Falls Camp	-16.66139	23.57280	929
PIPIDAE							
<i>Xenopus poweri</i>	BMNH 2018.5654	SL 2173	MK464274	Hillwood Farm	-11.26690	24.31666	1308
<i>Xenopus poweri</i>	BMNH 2018.5655	SL 2174	MK464273	Hillwood Farm	-11.26690	24.31666	1308
<i>Xenopus poweri</i>	BMNH 2018.5656	SL 2175	MK464272	Hillwood Farm	-11.26690	24.31666	1308

Appendix 1 (continued). List of amphibians and reptiles found in western Zambia, including species vouchers, GenBank accession numbers, and locality information. Museum acronym: BMNH – Natural History Museum, London, United Kingdom. GPS datum WGS-84.

Species	Voucher ID	Field ID	GenBank	Locality	Latitude	Longitude	Altitude
<i>Xenopus poweri</i>	BMNH 2018.5657	SL 2176	MK464271	Hillwood Farm	-11.26690	24.31666	1308
<i>Xenopus poweri</i>	BMNH 2018.5658	SL 2177		Hillwood Farm	-11.26690	24.31666	1308
<i>Xenopus poweri</i>	BMNH 2018.5659	SL 2222	MK464270	Nkwaji	-11.50420	24.56456	1386
<i>Xenopus pygmaeus</i>	BMNH 2018.5651	SL 2134	MK464269	Hillwood Farm	-11.26690	24.31666	1308
<i>Xenopus pygmaeus</i>	BMNH 2018.5652	SL 2135	MK464268	Hillwood Farm	-11.26690	24.31666	1308
<i>Xenopus pygmaeus</i>	BMNH 2018.5653	SL 2156	MK464267	Hillwood Farm	-11.26690	24.31666	1308
PTYCHADENIDAE							
<i>Ptychadena anchietae</i>	BMNH 2018.5735	SL 2040	MK464344	Itezhi-Tezhi, Kafue NP	-15.7734	26.01151	1036
<i>Ptychadena anchietae</i>	BMNH 2018.5736	SL 2039		Itezhi-Tezhi, Kafue NP	-15.7734	26.01151	1036
<i>Ptychadena anchietae</i>	BMNH 2018.5730	SL 2019		Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Ptychadena anchietae</i>	BMNH 2018.5731	SL 2020	MK464345	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Ptychadena anchietae</i>	BMNH 2018.5732	SL 2022		Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Ptychadena anchietae</i>	BMNH 2018.5733	SL 2023		Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Ptychadena anchietae</i>	BMNH 2018.5734	SL 2024		Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Ptychadena grandisonae</i>	BMNH 2018.5737	SL 2214	MK464318	Nkwaji	-11.5042	24.56456	1386
<i>Ptychadena grandisonae</i>	BMNH 2018.5738	SL 2215		Nkwaji	-11.5042	24.56456	1386
<i>Ptychadena grandisonae</i>	BMNH 2018.5739	SL 2226	MK464317	Nkwaji	-11.5042	24.56456	1386
<i>Ptychadena grandisonae</i>	BMNH 2018.5740	SL 2227	MK464316	Nkwaji	-11.5042	24.56456	1386
<i>Ptychadena grandisonae</i>	BMNH 2018.5741	SL 2228	MK464315	Nkwaji	-11.5042	24.56456	1386
<i>Ptychadena grandisonae</i>	BMNH 2018.5742	SL 2229	MK464314	Nkwaji	-11.5042	24.56456	1386
<i>Ptychadena grandisonae</i>	BMNH 2018.5743	SL 2230	MK464313	Nkwaji	-11.5042	24.56456	1386
<i>Ptychadena</i> cf. <i>guibei</i>	BMNH 2018.5764	SL 2035	MK464324	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Ptychadena</i> cf. <i>guibei</i>	BMNH 2018.5765	SL 2079	MK464323	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Ptychadena mapacha</i>	BMNH 2018.5772	SL 2014	MK464312	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Ptychadena</i> cf. <i>mossambica</i>	BMNH 2018.5754	SL 2042	MK464342	Itezhi-Tezhi, Kafue NP	-15.7734	26.01151	1036
<i>Ptychadena</i> cf. <i>mossambica</i>	BMNH 2018.5755	SL 2043	MK464341	Itezhi-Tezhi, Kafue NP	-15.7734	26.01151	1036
<i>Ptychadena</i> cf. <i>mossambica</i>	BMNH 2018.5756	SL 2044	MK464340	Itezhi-Tezhi, Kafue NP	-15.7734	26.01151	1036
<i>Ptychadena</i> cf. <i>mossambica</i>	BMNH 2018.5757	SL 2038	MK464339	Itezhi-Tezhi, Kafue NP	-15.7734	26.01151	1036
<i>Ptychadena</i> cf. <i>mossambica</i>	BMNH 2018.5763	SL 2021	MK464336	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Ptychadena</i> cf. <i>mossambica</i>	BMNH 2018.5758	SL 2088		Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Ptychadena</i> cf. <i>mossambica</i>	BMNH 2018.5759	SL 2089	MK464338	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Ptychadena</i> cf. <i>mossambica</i>	BMNH 2018.5760	SL 2094	MK464337	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Ptychadena</i> cf. <i>mossambica</i>	BMNH 2018.5761			Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Ptychadena</i> cf. <i>mossambica</i>	BMNH 2018.5753	SL 2041		Itezhi-Tezhi, Kafue NP	-15.7734	26.01151	1036
<i>Ptychadena nilotica</i>	BMNH 2018.5781	SL 2068	MK464327	Maramba Lodge, Livingstone	-17.8912	25.85821	900
<i>Ptychadena nilotica</i>	BMNH 2018.5773	SL 2058	MK464332	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Ptychadena nilotica</i>	BMNH 2018.5774	SL 2059	MK464331	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Ptychadena nilotica</i>	BMNH 2018.5775	SL 2061	MK464330	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Ptychadena nilotica</i>	BMNH 2018.5776	SL 2062		Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Ptychadena nilotica</i>	BMNH 2018.5777	SL 2278	MK464329	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Ptychadena nilotica</i>	BMNH 2018.5778	SL 2279		Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Ptychadena nilotica</i>	BMNH 2018.5779	SL 2280	MK464328	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Ptychadena nilotica</i>	BMNH 2018.5780	SL 2281		Nanzila Plains, Kafue NP	-16.28138	25.91676	1032

Herpetological survey of western Zambia

Appendix 1 (continued). List of amphibians and reptiles found in western Zambia, including species vouchers, GenBank accession numbers, and locality information. Museum acronym: BMNH – Natural History Museum, London, United Kingdom. GPS datum WGS-84.

Species	Voucher ID	Field ID	GenBank	Locality	Latitude	Longitude	Altitude
<i>Ptychadena obscura</i>	BMNH 2018.5768	SL 2155	MK464322	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena obscura</i>	BMNH 2018.5766	SL 2131	MK464311	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena obscura</i>	BMNH 2018.5767	SL 2132	MK464310	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena oxyrhynchus</i>	BMNH 2018.5783	SL 2250	MK464325	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena oxyrhynchus</i>	BMNH 2018.5782	SL 2060	MK464326	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Ptychadena porosissima</i>	BMNH 2018.5769	SL 2158	MK464321	Hillwood Farm	-11.27531	24.31977	1340
<i>Ptychadena porosissima</i>	BMNH 2018.5770	SL 2168	MK464320	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena porosissima</i>	BMNH 2018.5771	SL 2248	MK464319	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena</i> cf. <i>taenioscelis</i>	BMNH 2018.5784	SL 2169	MK464335	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena</i> cf. <i>taenioscelis</i>	BMNH 2018.5785	SL 2171	MK464334	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena</i> cf. <i>taenioscelis</i>	BMNH 2018.5786	SL 2172	MK464333	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena upembae</i>	BMNH 2018.5750	SL 2154	MK464348	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena upembae</i>	BMNH 2018.5751	SL 2170	MK464347	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena upembae</i>	BMNH 2018.5752	SL 2251	MK464346	Hillwood Farm	-11.2669	24.31666	1308
<i>Ptychadena upembae</i>	BMNH 2018.5744	SL 2192	MK464354	Nkwaji	-11.60592	24.55448	1244
<i>Ptychadena upembae</i>	BMNH 2018.5745	SL 2193	MK464353	Nkwaji	-11.60592	24.55448	1244
<i>Ptychadena upembae</i>	BMNH 2018.5746	SL 2194	MK464352	Nkwaji	-11.60592	24.55448	1244
<i>Ptychadena upembae</i>	BMNH 2018.5747	SL 2212	MK464351	Nkwaji	-11.56594	24.52659	1311
<i>Ptychadena upembae</i>	BMNH 2018.5748	SL 2218	MK464350	Nkwaji	-11.53906	24.55262	1336
PYXICEPHALIDAE							
<i>Amietia chapini</i>	BMNH 2018.5660	SL 2186	MK464482	Hillwood Farm	-11.26690	24.31666	1308
<i>Amietia chapini</i>	BMNH 2018.5661	SL 2185	MK464481	Hillwood Farm	-11.26690	24.31666	1308
<i>Amietia chapini</i>	BMNH 2018.5662	SL 2178		Hillwood Farm	-11.26690	24.31666	1308
<i>Amietia chapini</i>	BMNH 2018.5663	SL 2183	MK464480	Hillwood Farm	-11.26690	24.31666	1308
<i>Amietia chapini</i>	BMNH 2018.5664	SL 2151		Hillwood Farm	-11.26690	24.31666	1308
<i>Amietia chapini</i>	BMNH 2018.5665	SL 2167		Hillwood Farm	-11.26690	24.31666	1308
<i>Pyxicephalus</i> cf. <i>adspersus</i>	BMNH 2018.5787	SL 2077	MK464309	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Pyxicephalus</i> cf. <i>adspersus</i>	BMNH 2018.5788	SL 2082	MK464308	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Pyxicephalus</i> cf. <i>adspersus</i>	BMNH 2018.5789	SL 2083	MK464307	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Pyxicephalus</i> cf. <i>adspersus</i>	BMNH 2018.5790	SL 2084	MK464306	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Pyxicephalus</i> cf. <i>adspersus</i>	BMNH 2018.5791	SL 2097	MK464305	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Tomopterna marmorata</i>	BMNH 2018.5792	SL 2070	MK464287	Maramba Lodge, Livingstone	-17.89120	25.85821	900
<i>Tomopterna</i> sp.	BMNH 2018.5793	SL 2078	MK464286	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Tomopterna</i> sp.	BMNH 2018.5794	SL 2085	MK464285	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Tomopterna</i> sp.	BMNH 2018.5795	SL 2086	MK464284	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Tomopterna</i> sp.	BMNH 2018.5796	SL 2092	MK464283	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Tomopterna</i> sp.	BMNH 2018.5797	SL 2093	MK464282	Sioma Ngwezi NP	-16.89873	23.59847	1009
RHACOPHORIDAE							
<i>Chiromantis xerampelina</i>	BMNH 2018.5798	SL 2036	MK464456	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
REPTILIA: SQUAMATA							
AGAMIDAE							
<i>Agama armata</i>	BMNH 2018.2751	SL 2253	MK464483	Ngonye Falls Camp	-16.66139	23.57280	929

Appendix 1 (continued). List of amphibians and reptiles found in western Zambia, including species vouchers, GenBank accession numbers, and locality information. Museum acronym: BMNH – Natural History Museum, London, United Kingdom. GPS datum WGS-84.

Species	Voucher ID	Field ID	GenBank	Locality	Latitude	Longitude	Altitude
CHAMAELEONIDAE							
<i>Chamaeleo dilepis</i>	BMNH 2018.2755	SL 2099	MK464458	Chavuma FR	-13.07006	22.92880	1073
<i>Chamaeleo dilepis</i>	BMNH 2018.2756	SL 2100	MK464457	Chavuma FR	-13.07006	22.92880	1073
COLUBRIDAE							
<i>Crotaphopeltis hotamboeia</i>	BMNH 2018.2773	SL 2247	MK464455	Hillwood Farm	-11.26690	24.31666	1308
<i>Crotaphopeltis hotamboeia</i>	BMNH 2018.2776	SL 2272		Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Crotaphopeltis hotamboeia</i>	BMNH 2018.2777	SL 2258	MK464454	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Rhamnophis aethiopissa ituriensis</i>	BMNH 2018.2772	SL 2249	MK464304	Hillwood Farm	-11.26690	24.31666	1308
<i>Philothamnus hoplogaster</i>	BMNH 2018.2775	SL 2277	MK464398	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Thelotornis kirtlandii</i>	BMNH 2018.2760	SL 2182		Hillwood Farm	-11.26690	24.31666	1308
GEKKONIDAE							
<i>Hemidactylus mabouia</i>	BMNH 2018.2740	SL 2264		Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Hemidactylus mabouia</i>	BMNH 2018.2742	SL 2263	MK464452	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Hemidactylus mabouia</i>	BMNH 2018.2741	SL 2276	MK464453	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Lygodactylus angolensis</i>	BMNH 2018.2766	SL 2213	MK464405	Nkwaji	-11.52743	24.53532	1343
<i>Lygodactylus angolensis</i>	BMNH 2018.2767	SL 2187	MK464404	Nkwaji	-11.60592	24.55448	1244
<i>Lygodactylus chobiensis</i>	BMNH 2018.2743	SL 2267	MK464403	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Lygodactylus chobiensis</i>	BMNH 2018.2744	SL 2268		Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Pachydactylus punctatus</i>	BMNH 2018.2757	SL 2265	MK464400	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Pachydactylus punctatus</i>	BMNH 2018.2758	SL 2266		Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Pachydactylus punctatus</i>	BMNH 2018.2759	SL 2269		Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
GERRHOSAURIDAE							
<i>Gerrhosaurus bulsi</i>	BMNH 2018.2754	SL 2181		Hillwood Farm	-11.26690	24.31666	1308
LACERTIDAE							
<i>Ichnotropis capensis</i>	BMNH 2018.2746	SL 2103	MK464417	Chavuma FR	-13.07006	22.92880	1073
<i>Ichnotropis capensis</i>	BMNH 2018.2747	SL 2260	MK464416	Lukwakwa	-12.66084	24.43697	1063
<i>Ichnotropis capensis</i>	BMNH 2018.2749	SL 2259		Lukwakwa	-12.66084	24.43697	1063
<i>Ichnotropis capensis</i>	BMNH 2018.2748	SL 2274		Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Ichnotropis capensis</i>	BMNH 2018.2750	SL 2273	MK464415	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Ichnotropis capensis</i>	BMNH 2018.2745	SL 2256	MK464418	Sioma Ngwezi NP	-16.89873	23.59847	1009
<i>Meroles squamulosus</i>	BMNH 2018.2753	SL 2275	MK464401	Nanzila Plains, Kafue NP	-16.28138	25.91676	1032
<i>Meroles squamulosus</i>	BMNH 2018.2752	SL 2257	MK464402	Sioma Ngwezi NP	-16.89873	23.59847	1009
LAMPROPHIIDAE							
<i>Atractaspis congica</i>	BMNH 2018.2274	SL 2160	MK464459	Hillwood Farm	-11.27444	24.32444	1416
SCINCIDAE							
<i>Typhlacontias rohani</i>	BMNH 2018.2761	SL 2254	MK464275	New HQ complex of Sioma Ngwezi NP	-16.66953	23.56743	999
<i>Panaspis</i> cf. <i>wahlbergi</i>	BMNH 2018.2738	SL 2101	MK464399	Chavuma FR	-13.07006	22.92880	1073
<i>Panaspis</i> cf. <i>wahlbergi</i>	BMNH 2018.2739	SL 2104		Chavuma FR	-13.07006	22.92880	1073
<i>Trachylepis</i> cf. <i>albopunctata</i>	BMNH 2018.2762	SL 2270	MK464281	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Trachylepis</i> cf. <i>albopunctata</i>	BMNH 2018.2763	SL 2032		Mayukuyuku, Kafue NP	-14.91533	26.06311	1012

Herpetological survey of western Zambia

Appendix 1 (continued). List of amphibians and reptiles found in western Zambia, including species vouchers, GenBank accession numbers, and locality information. Museum acronym: BMNH – Natural History Museum, London, United Kingdom. GPS datum WGS-84.

Species	Voucher ID	Field ID	GenBank	Locality	Latitude	Longitude	Altitude
<i>Trachylepis</i> cf. <i>albopunctata</i>	BMNH 2018.2765	SL 2013	MK464280	Mayukuyuku, Kafue NP	-14.91533	26.06311	1012
<i>Trachylepis damarana</i>	BMNH 2018.2764	SL 2255	MK464279	New HQ complex of Sioma Ngwezi NP	-16.66953	23.56743	999
<i>Trachylepis wahlbergii</i>	BMNH 2018.2769	SL 2271	MK464278	Itezhi-Tezhi, Kafue NP	-15.77340	26.01151	1036
<i>Trachylepis wahlbergii</i>	BMNH 2018.2768	SL 2262		Lukwakwa	-12.66084	24.43697	1063
<i>Trachylepis wahlbergii</i>	BMNH 2018.2770	SL 2261	MK464277	Lukwakwa	-12.66084	24.43697	1063
<i>Trachylepis wahlbergii</i>	BMNH 2018.2771	SL 2188	MK464276	Nkwaji	-11.60592	24.55448	1244
REPTILIA: Testudines							
TESTUDINIDAE							
<i>Kinixys spekii</i>	No voucher	No voucher		Nkwaji	-11.53906	24.55262	1336



Hiding in the bushes for 110 years: rediscovery of an iconic Angolan gecko (*Afrogecko ansorgii* Boulenger, 1907, Sauria: Gekkonidae)

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Abstract.—Boulenger (1907) described a new gecko '*Phyllodactylus*' *ansorgii* based on two adult females from 'Maconjo, Benguella, Angola,' but subsequent taxonomic reviews of leaf-toed geckos ascribed southern African lineages to new genera and this species has since been tentatively placed under '*Afrogecko*.' For over 110 years the type locality remained a mystery, and the gecko became a lost icon of Angolan herpetology. Early searches for the gecko were confounded by misinterpretation of the type locality 'Maconjo,' which it is now evident was confused with a toponym that is a well-known historical locality. Following the discovery of new material in coastal Benguela, an examination of historical documents and cartographic material allowed the original collecting area for the type material to be identified. Specific surveys in this area resulted in the collection of topotypic material and the recording of behavioral observations and notes on the ecology of the species. *Afrogecko ansorgii* is a slender, gracile, and arboreal gecko that inhabits small bushy trees, particularly blackthorn (*Senegalia mellifera* subsp. *detinens*), in the arid coastal scrubland of the Benguela coastal region. All *A. ansorgii* have been found in or nearby blackthorn in which the activity of termites (Kalotermitidae) has created hollow stems in which the geckos shelter. The type locality for *Phyllodactylus* (= *Afrogecko*) *ansorgii* Boulenger, 1907 and *Mabuia* (= *Trachylepis*) *laevis* Boulenger, 1907, both described at the same time from 'Maconjo, Benguella' is accordingly restricted, and topotypic material for the latter species was also obtained.

Key Words. Ansorge, blackthorn, Kaokoveld, Maconjo, Reptilia, type locality, *Trachylepis laevis*

Resumo.—Boulenger (1907) descreveu a nova osga '*Phyllodactylus*' *ansorgii* a partir de duas fêmeas oriundas de 'Maconjo, Benguella, Angola,' mas subseqüentes revisões taxonómicas das osgas-de-dedos-de-folha fez corresponder a novos géneros as linhagens da África austral e desde então esta espécie tem sido tentativamente incluída em '*Afrogecko*.' Durante mais de 110 anos a localidade típica permaneceu um mistério, e a osga tornou-se um ícone perdido da herpetologia Angolana. Buscas iniciais pela osga ficaram baralhadas devido a uma má interpretação da localidade típica 'Maconjo,' que é hoje evidente ter sido confundida com um topónimo que é uma localidade histórica bem conhecida. Após a descoberta de novo material na região costeira de Benguela, a despistagem de documentos históricos e material cartográfico permitiu identificar a área de colheita original do material típico. Levantamentos específicos nesta área resultaram na colheita de material topotípico e no registo de observações comportamentais e notas sobre a ecologia da espécie. O *Afrogecko ansorgii* é uma osga delgada, delicada e arborícola que habita pequenas árvores de porte arbustivo, particularmente espinheiras (*Senegalia mellifera* subsp. *detinens*), nas estepes áridas arbustivas da região costeira de Benguela. Todos os *A. ansorgii* foram encontrados nestas espinheiras ou na sua vizinhança, e onde a actividade de térmitas (Kalotermitidae) gerou talos ocos onde as osgas se abrigam. A localidade típica para o *Phyllodactylus* (= *Afrogecko*) *ansorgii* Boulenger 1907 e para o *Mabuia* (= *Trachylepis*) *laevis* Boulenger 1907, ambos descritos na mesma altura para 'Maconjo, Benguella', é desta forma restringida, e material topotípico da última espécie foi também obtido.

Palavras-chave: Reptilia, localidade típica, Maconjo, Ansorge, espinheira, Kaokoveld, *Trachylepis laevis*

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Introduction

The biodiversity of Angola, and particularly that of the herpetofauna, is acknowledged to be inadequately known (Branch 2016; Huntley and Ferrand 2019). This was not always the case, and like many countries in Africa the early phase of colonial exploration and settlement resulted in the discovery of numerous novel animals. For almost 50 years (1866–1915) these collections were sent to European museums for study and description, and they stimulated much scientific interest. Early studies on the Angolan herpetofauna were numerous, particularly those of José Vicente Barbosa du Bocage, the father of Angolan herpetology. His monographic *Herpétologie d'Angola et du Congo* (Bocage 1895) remained for over a century the definitive synthesis of the country's herpetofauna, until the recent publication of a historic herpetological atlas (Marques et al. 2018). Currently, studies on the herpetofauna of Angola are entering a new phase following the closure of protracted hostilities at the end of the colonial era. Recent field surveys (e.g., Huntley 2009; Ernst et al. 2014; Huntley and Francisco 2015; Ceriaco et al. 2016, 2018; Conradie et al. 2016) have uncovered new species of amphibians (Conradie et al. 2012a, 2013; Ceriaco et al. 2018) and reptiles (Conradie et al. 2012b; Stanley et al. 2016). However, a number of important historical species described during the colonial period still remain known from only their original description, e.g., *Sepsina copei* Bocage, 1866 and *Cordylus angolensis* (Bocage 1895), or only a few subsequent specimens, e.g., *Monopeltis luandae* Gans 1976 (Branch et al. 2018), *Psammophis ansorgii* Boulenger 1905, and *Psammophylax ocellatus* Bocage 1873 (Branch et al. 2019a). Tragically, much of the historical material studied by Bocage, including almost all of his type material, was lost in the fire that destroyed the Museu Bocage collections in 1978.

Perhaps the most iconic of the 'lost' Angolan species is Ansorge's Leaf-toed Gecko, which was described by Boulenger (1907) as *Phyllodactylus ansorgii*, over 110 years ago. Leaf-toed geckos have a globally wide-ranging distribution and all of them used to be included within *Phyllodactylus* until a comprehensive review performed by Bauer (1997) ascribed the southern African species to three new genera. The genus *Afrogecko* initially included two species found in South Africa, *A. porphyreus*, and *A. swartbergensis*, and two Angolan leaf-toed geckos, *A. plumicaudus* and *A. ansorgii* (Bauer 1997; Haacke 2008). However, more recent phylogenetic studies revealed deeply independent evolutionary histories among these lineages, leading *A. swartbergensis* and *A. plumicaudus* to be transferred to the new monotypic genera *Ramigecko* and *Kolekanos*, respectively, while due to the lack of available material *ansorgii* was provisionally maintained within *Afrogecko* (Heinicke et al. 2014).

The species description was based on two female specimens from Maconjo, Benguella (now Benguela) collected by Dr. W.J. Ansorge on one of his Angolan journeys, between 1903 and 1906, and the types, BMNH 1946.8.24.52–53, were deposited in the Natural History Museum London (United Kingdom). The exact locality

of Ansorge's Maconjo has caused confusion, and erroneous interpretation of its whereabouts probably delayed the species' rediscovery. Moreover, the species is now known to live in an unusual habitat, and this may have also led to it being overlooked. Crawford-Cabral and Mesquitela (1989) summarized all known localities for Angolan terrestrial vertebrates and considered Maconjo a variant spelling of Maconge, an old farm situated in Namibe Province, formerly Mossamedes (= Moçâmedes) District (15°01'S, 13°12'E, 700 m asl). Maconjo, Mossamedes features as a collecting locality for reptiles in the 19th century for the famous Portuguese collector José de Anchieta (de Andrade 1985), and in his monograph Bocage (1895) recorded numerous reptiles from Maconjo, Mossamedes, including: the terrapin *Pelomedusa galeata* (= *P. subrufa*); the lacertids *Nucras tessellata* and *Eremias lugubris* (= *Heliobolus lugubris*); the rupicolous skink *Mabuia chimbana* (= *Trachylepis chimbana*); the python *Python natalensis*; the snakes *Prosymna frontalis* (= *P. angolensis*, *fide* Broadley 1980), *Psammophylax nototaenia* (= *Hemirhaggheris viperina*, *fide* Broadley 2000), *Psammophis sibilans* (= *P. subtaeniatus*), *Elapsoidea guentheri* var. *semiannulata* (= *E. s. semiannulata*), and *Causus resimus*. Many of these have been subsequently recorded in the region (data not shown).

A farm and stream at the base of the Humpata plateau near Leba Pass are called Maconjo on old maps and lie in the vicinity of the old Portuguese fort of Capangombe, which dates from the mid-nineteenth century and marks the route for some of the earliest inland incursions of colonists in southern Angola. This locality was then included in the District of Mossamedes. However, Boulenger referred the gecko's type locality to 'Maconjo, Benguella,' placing it in Benguela instead of Mossamedes District. This suggests either that it was ascribed by Ansorge to the wrong district, or that there could exist two different sites bearing the name Maconjo. It should be noted that mistaking Mossamedes for Benguela would have been unlikely in the early 20th century, when both districts were well established and had been for some time. They have also remained as separate administrative entities ever since. Yet this incongruence seems to have gone unnoticed, and it appears that most subsequent researchers have considered there was only one Maconjo, and the references to different provinces, Mossamedes (= Namibe) or Benguela, were either ignored or taken as synonyms (Bauer et al. 1997; Heinicke et al. 2014; Ceriaco et al. 2016; Uetz et al. 2018). The accepted wisdom that there was only one Maconjo, and that it was situated below the escarpment in the current Namibe Province, resulted in numerous unsuccessful searches for the gecko around Capangombe by various researchers from 1974 to 2016. More recently, Maconjo was tentatively synonymized with another well-known collecting site in southwestern Angola 'Fazenda Mucungo' (Marques et al. 2018), however, no further details were provided to justify the new locality. Mucungo also lies on the coastal plain of Namibe and therefore cannot resolve the geographical discrepancies.

Another species from 'Maconjo' that Boulenger (1907) described from the same Ansorge collection

was the small rupicolous skink *Mabuia* (= *Trachylepis*) *laevis*, commonly called the Angolan Blue-tailed Skink (*T. laevis*). It is rupicolous, sheltering in thin cracks in hard, fractured rocks and has numerous morphological adaptations to this habitat (Paluh and Bauer 2017). Despite its conspicuous coloration, the species has only rarely been collected in Angola after the original description (Boulenger 1907). Laurent (1964) recorded a male from Munhino, 50 km W of Lubango, and Hellmich (1957) reported a problematic specimen from Piri-Dembos (= Piri) that should be treated with caution (Laurent 1964; Ceriaco et al. 2016). The Angolan Blue-tailed Skink was recently recorded from granite outcrops 50 km east of Namibe (W.R. Branch, pers. comm.), and just north of Tambor near Iona National Park (Ceriaco et al. 2016), and additional material has been collected in northern Namibe Province, at Chapéu Armado, Serra da Neve, and Lola by the authors of the current study. Still, the most significant collections of *T. laevis* were made by Wulf Haacke during an expedition to southwestern Angola in 1971, when he collected 18 specimens from Tambor and other localities in Namibe Province (all deposited in the Ditsong Natural History Museum, Pretoria, South Africa). In addition, and importantly, he collected the first records since Ansorge from Benguela Province, with material from 35 km south of Dombe Grande and 53 km south of Benguela.

Until now, no additional material for *A. ansorgii* had ever been collected, despite searches by experienced herpetologists in the 1970s and after 2009. In this paper we report on finding the species and obtaining new material that includes the first male specimens. The type locality is here confidently restricted, and topotypic material was collected for both *A. ansorgii* and *T. laevis*. In addition, by collecting the gecko on a second site, we extend its known range by about 100 km. Behavioral and habitat observations were also recorded, and provide useful insights into their unique ecological requirements.

Materials and Methods

To investigate local toponyms and locate the type locality, historical cartographic material was thoroughly examined and, in the absence of available diaries for Dr. Ansorge's Angolan journeys, we consulted a remarkable manuscript by the American ornithologist James Chapin which provides a detailed summary of Ansorge's itineraries in Angola (Chapin, undated).

After first encountering *A. ansorgii*, we conducted a series of additional field trips in search of the gecko between November 2016 and June 2018, to five different sites, including the restricted type locality and additional locations where we looked for their presence. The surveys included mostly nocturnal searches, and daytime habitat observations. A total of 23 specimens of *A. ansorgii* were collected in two sites, including the restricted type locality. All specimens were photographed in life, and subsequently preserved in formalin. Collected specimens were deposited at Port Elizabeth Museum (PEM R23907–23916), at ISCED – Instituto Superior de Ciências da Educação da Huíla (NB603–605; NB822–826) and at Kissama Foundation (KFH0007–0010). Additionally,

behavioral observations were also recorded by locating the geckos at night with flashlights and observing their foraging habits, and by confirming their daytime shelters. Reptile species lists were compiled for each site visited, and topotypic material was also secured for *T. laevis*, with one specimen collected and deposited at Kissama Foundation collection (FKH0018).

Results

Type locality: From Chapin's summary it is apparent that Ansorge visited 'Makonjo, Benguella' on 7 July 1904. Chapin used the spelling 'Makonjo' instead of following Boulenger's 'Maconjo,' but the use of a 'k' or 'c' is often interchangeable when applied to Angolan toponymy. To avoid continued confusion with toponyms we hereafter keep the spelling as Maconjo and add the district/province name when relevant. Ansorge left the locality of 'Huxe, Benguella' (= Uche, 12°44'S, 13°21'E, 250 m asl) on the previous day on his way to Benguela's sandpits and the town of Catengue (13°02'S, 13°44'E, 550 m asl), which he reached on 11 July 1904. This route, depicted on a map included in the manuscript, places the locality of Maconjo clearly in Benguela as opposed to Namibe's coastal plain, and within a few days travel on foot from Benguela town. In addition, there are no other records of localities of similar spelling in the manuscript, and no suggestion that Ansorge ever visited Capangombe region. Moreover, based on Chapin's document it appears that Ansorge never descended the escarpment from the Humpata plateau, and although he referred to his 1906 route south of Caconda as being in Mossamedes District, Chapin failed to recognize the then newly created Huíla District. As a result, there is no evidence to suggest that Ansorge even collected in the former Mossamedes District as it was defined in his time, and which broadly corresponds to the current Namibe Province.

Comparing Chapin's notes with cartographic material allowed the reconstruction of Ansorge's probable route (Fig. 1) and the identification along it of a region by the name of 'Conjo' (12°53'S, 13°24'E) in Benguela Province. This area, approximately 20 km south of Uche and on the route towards Catengue, lies between 300 and 450 m asl, and includes two small north-flowing dry sandy streams named respectively as 'Conjo' and 'Conjo Pequeno,' both tributaries to another ephemeral stream named 'Cocumba.' The region is in Benguela's coastal plain and would be realistically within one-day's reach from Uche on foot, and enroute to Catengue, making it extremely likely that it corresponds to the true type locality of 'Maconjo, Benguella' as recorded by Ansorge (in Boulenger 1907). Interestingly, in the local language Conjo means spoor and the prefix 'Ma-' is commonly used to signify plurality, so the toponymy Maconjo can be appropriately interpreted as 'the site of various Conjos' or 'site of many spoor.'

On the basis of Chapin's notes and cartographic material we here restrict the type locality of *Phyllodactylus* (= *Afrogecko*) *ansorgii* and *Mabuia* (= *Trachylepis*) *laevis*, both described by Boulenger (1907) from 'Maconjo, Benguella,' to the vicinity of the streams Conjo, Conjo Pequeno, and Cocumba (12°52'S, 13°21'E, 355 m asl),

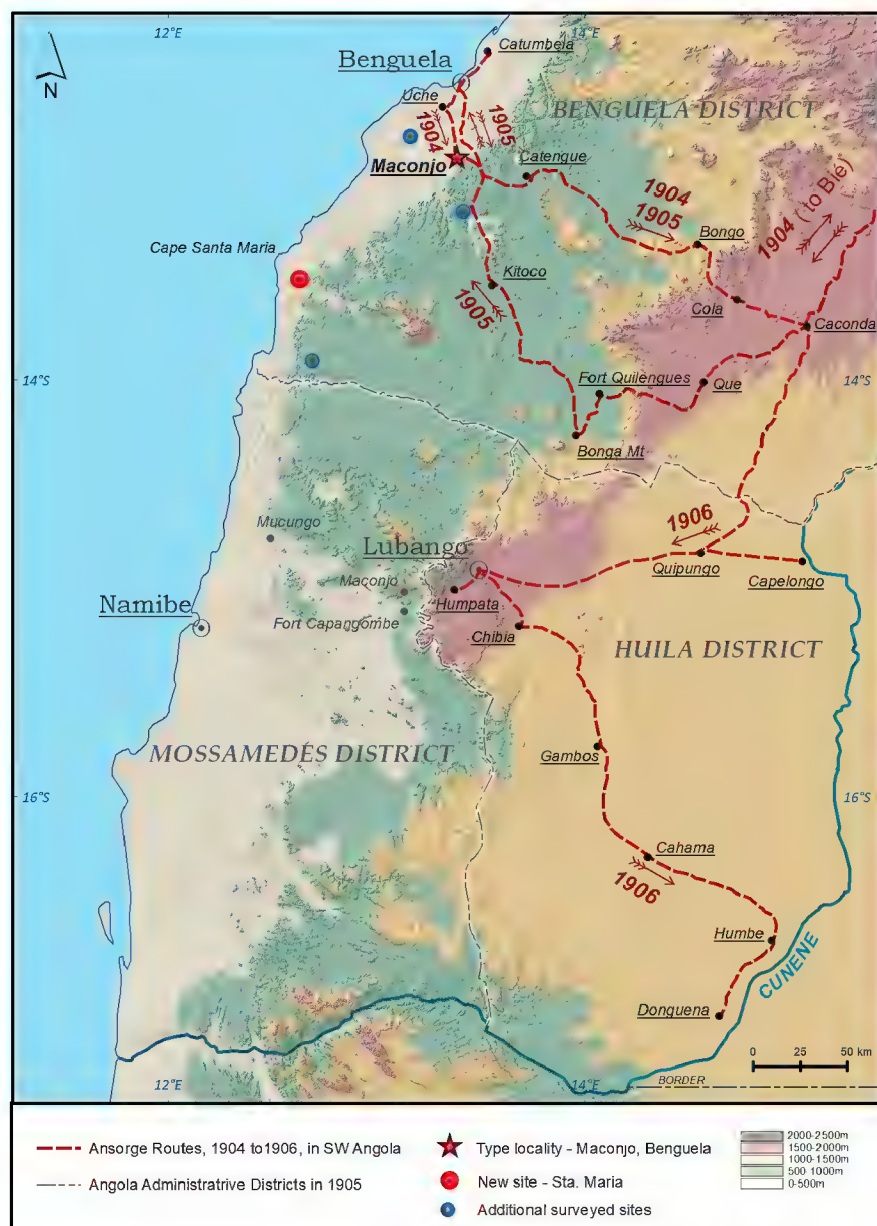


Fig. 1. Travel routes in Angola by Dr. Ansorge between 1904 and 1906, as per Chapin's manuscript. Former district (broadly corresponding to current provinces) borders are shown, as well as collecting sites and other relevant localities.

20 km south of Uche, Benguela Province, Angola (Fig. 1). Both species, *Afrogecko ansorgii* and *Trachylepis laevis*, were collected at this site (see below).

Rediscovery: The gecko was first rediscovered 135 km southwest of Benguela town on 12 November 2016. The site is located about three km from the Lucira-Benguela road, at the head of a valley leading down to Cape Sta Maria, Benguela Province (13°31'S, 12°38'E, 288 m asl). The locality is ca. 185 km northwest of Maconjo, Namibe, and ca. 100 km southwest of the type locality in Benguela Province (Fig. 1). A total of 10 specimens were collected at night whilst foraging in the branches of thorny bushes (Fig. 2), mainly of blackthorn *Senegalia mellifera detinens*. Subsequently, three additional specimens were collected, and nine others observed in the same habitat and under similar circumstances, at two other nearby sites surveyed five km and three km to the northwest, on 12–13 July 2017 and 11–12 August 2017, respectively. Most specimens were located at night on bushes situated at the base or mid-slopes of granitic hills, and although a few were observed in various species of small trees, the majority were found among the spiny branches of blackthorn bushes. One specimen was found during the day sheltering inside a hollow blackthorn branch (Fig. 3A).

After the cartographic identification of the potentially true type locality, specific visits to the Conjo area to search for geckos were made on 25–26 November 2017

and 5 December 2017. The region surveyed was located approximately half-way between the towns of Benguela (35 km) and Catengue (47 km) and five km south of the small village of Talamajamba. As it shared some features with sites at Sta Maria, although lacking in rocky habitat suitable for *Trachylepis laevis*, we first focused on a site located on a sand road that crosses the Cocumba dry stream and runs a few hundred meters parallel to the east of Conjo Pequeno (12°54'S, 13°24'E, 395 m asl). The searches resulted in our obtaining topotypic material and further observations. Broadening the search, additional visits on 12–13 March 2018 and 5 June 2018, focused on an area around three isolated granite outcrops present along the stream Cocumba and a mere 500 m west of the confluence with stream Conjo (12°52'S, 13°21'E, 355 m asl). Although the Cocumba is a dry stream on a semi-desert environment, local pastoralists have long dug artisanal wells to gain access to water on the river bed at the base of the granite outcrops. The presence of these wells on the confluence of Conjo and Cocumba streams offers a realistic scenario for the site 'Maconjo' to have been used as a stopover locality between Benguela and Catengue by Ansorge in 1904. Here, additional specimens of *A. ansorgii* were found in nearby bushes, and a specimen of *T. laevis* was collected in the granite outcrops, constituting the first topotypic material for this species.

At Maconjo, a total of 10 specimens of *A. ansorgii* were collected and 14 additional individuals were observed foraging at night in bushes over the four visits, mostly found in *Senegalia mellifera detinens*, but also in *Terminalia prunioides*, *Commiphora cf. africana*, and *Salvadora persica*, and once on a dry grass stem. Most geckos were spotted in the early evening (approximately between 19h00 and 21h00) in small branches 1–2 m above ground. One specimen was observed partially exiting a hollow blackthorn branch shortly after sunset, as exemplified in a photograph (Fig. 3B).

Three additional coastal sites (Fig. 1) were also surveyed in search of the geckos, one was an intermediate location between Maconjo and Sta Maria, at Chimalavera Regional Park (12°50'S, 13°10'E, 255 m asl), on a sandy plateau over an east-facing limestone ridge, Benguela Province; another ca. 35 km south of Maconjo and near the Coporolo River (13°12'S, 13°25'E, 380 m asl); and one further south at 17 km W of Chicambi Village, on sandy flats with a granite ridge, in Namibe Province (13°55'S, 12°41'E, 531 m asl). In these sites the habitat was similar to that at Cape Sta Maria, with numerous *Senegalia mellifera detinens* bushes on sandy soil and with adjacent rock outcrops. However, in all these three cases the blackthorn bushes lacked hollow branches and no geckos were observed during nocturnal searches.

Habitat observations: On the vegetation map of Angola, Barbosa (1970) defines a very extensive region as semi-desert coastal steppe, starting as a narrow coastal strip around 11° south, then becoming broader inland towards the southern border with Namibia. However, this is a very wide and rough classification and includes a transition from dry spiny savannahs dominated by acacias (*Senegalia* spp.) to mopane woodlands characterized

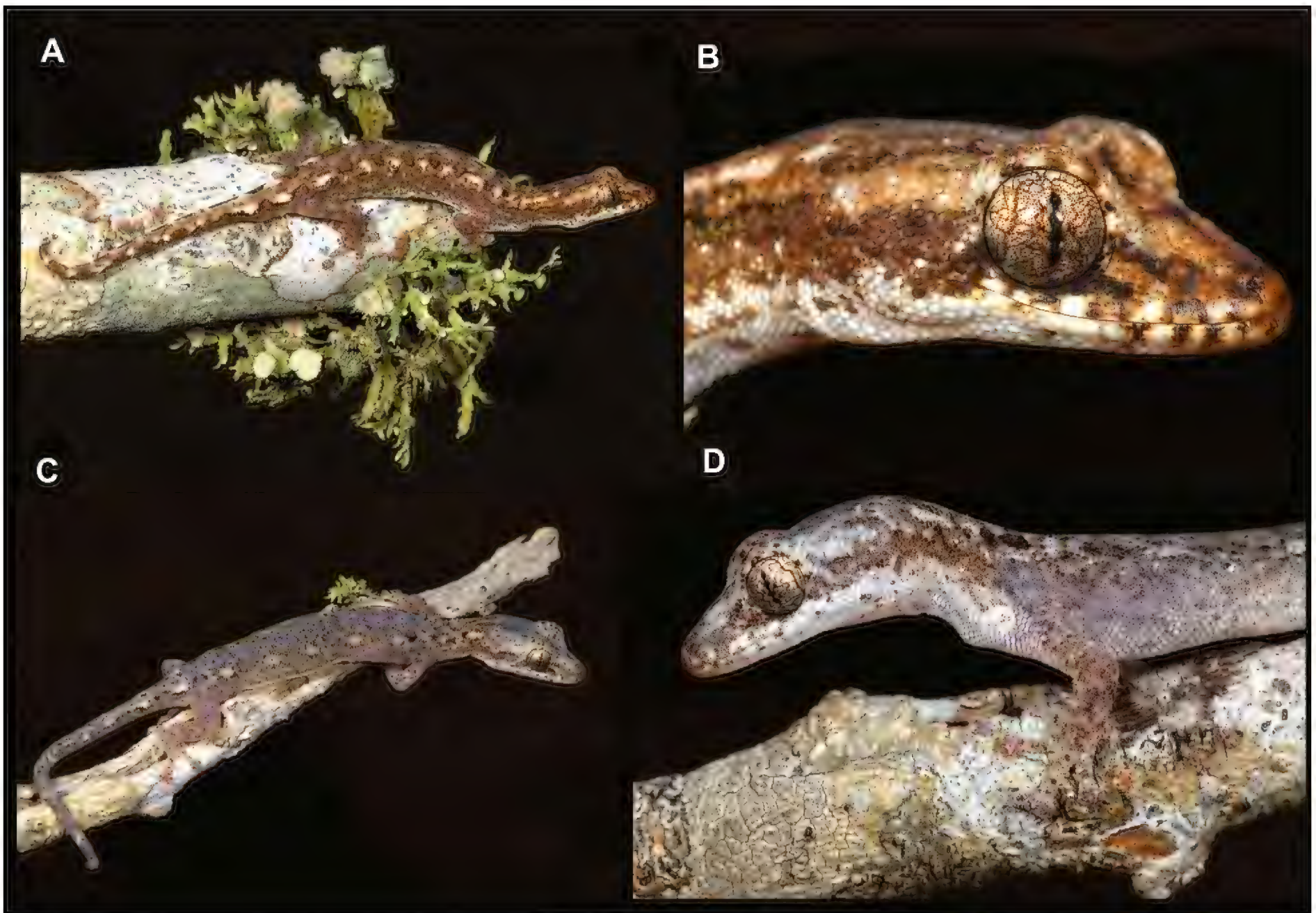


Fig. 2. *Afrogecko ansorgii*: adult male, PEM R23907 (in life). **(A)** Whole body on small branch of *Senegalia mellifera detinens* with epiphytic lichen. **(B)** Close up of head of same adult male. **(C)** *Afrogecko ansorgii*: adult female, PEM R23912 (in life) on small branch of *Senegalia mellifera detinens*. **(D)** Close up of head of same adult female. Note elongate, subcylindrical body, relatively short cylindrical tail that is shorter than SVL and partially prehensile, long toes with expanded terminal scansors, predominantly dark brown dorsal coloration, and dorsolateral series of irregular pale blotches that extend on to the tail.

by the dominance of *Colophospermum mopane*. Still, he refers to the arid regions near Benguela as being characterized by an abundance of *Senegalia mellifera detinens*, *Senegalia* spp., *Salvadora persica*, *Euphorbia* spp., and *Boscia* spp. (Barbosa 1970) which likely correspond to the more relevant areas for Ansorge's Gecko.

At Maconjo, Benguela, the restricted type locality, the terrain is relatively flat, with sandy soils, irregularly interspersed with small granitic rocks, and with a few isolated granite outcrops at the Cocumba stream (Fig. 4A). The vegetation was relatively dense and diverse, the trees often in close proximity or touching, but with low canopies and not growing larger than the size of a bush (Fig. 4B). The dominant tree/bush species were *Senegalia mellifera detinens* and *Terminalia prunioides*, but other species also found to be common were *Commiphora* cf. *africana*, *Boscia microphylla*, *Vachellia reficiens*, and *Salvadora persica*. Succulents were also recorded, notably the conspicuous *Aloe littoralis*, plus a few *Hoodia* cf. *parviflora* and the invasive *Opuntia ficus-indica*.

The Sta Maria site is situated at a slightly lower elevation, being closer to the coast and further south than the type locality, and in a more arid coastal environment. It possibly receives lower rainfall, but this may be

compensated for by the higher incidence of coastal fog which is a feature of the whole Namib Desert coastline (Cermak 2012). The effect of regular fogs, which may extend well inland, is evident in the local abundance of lichens that often cover the branches of bushes (Figs. 2A, 4C). At this locality the vegetation was much sparser than at Maconjo, Benguela, particularly in the sandy valleys, but the topography was often much more varied with large granite boulders and steep granitic hills (Fig. 4D). *Senegalia mellifera detinens* was the most abundant tree, while other common species recorded were *Salvadora persica*, *Commiphora multijuga*, *Phaeoptilum spinosum*, and *Terminalia prunioides*.

A striking find that appears positively linked with the occurrence of the gecko is the existence in both sites of abundant hollow branches in *Senegalia mellifera detinens* caused by the activity of termites (Kalotermitidae) [Fig. 5]. By eating the heartwood of dead branches and leaving the outer wood and bark intact, the termites hollow out branches, making small exit holes at the branching nodes. This activity creates ideal daytime shelters for *Afrogecko ansorgii*, with multiple entry and exit points. These hollow branches subsequently break off and accumulate below the tree, where they may still be used as refugia by the geckos.

Table 1. Variation observed for *Afrogecko ansorgii*, comparing the type series from Maconjo, with new series obtained in Maconjo and Sta Maria. Continuous measurements in mm (mean and range) for TL= total length, SVL= snout-vent length and Tail. Meristic and qualitative variation (median and range) is given for number of precloacal pores and cloacal spurs.

	TL	SVL	Tail	Pores	Spurs
Type Series – Maconjo					
Females (n=2)	7–5	45	30	0	na
New Series – Maconjo					
Females (n=5)*	75.1 (71.4–79.0)	43.9 (41.3–45.8)	31.3 (30.1–33.2)	0	1
Males (n=4)	65 (57.8–68.8)	38.2 (34.4–40.6)	26.8 (23.4–29.0)	8 8–9	2 2
Juvenile Female (n=1)	68	38.6	29.4	0	1
New series – Sta Maria					
Females (n=5)**	63.3 (56.2–69.3)	39.4 (34.7–42.9)	23.8 (22.0–26.4)	0	0
Males (n=6)**	60 (53.2–63.5)	35.7 (34.4–38.0)	24.2 (18.8–27.5)	9 7–9	1 1–2
Juvenile Female (n=1)	54.4	32.3	22.1	0	0

* For Tail and TL measurements n=3 due to truncated tails; ** For Tail and TL measurements n=4 due to truncated tails.

Natural history: The geckos were mostly first seen lying flat or moving slowly on thin branches in the early hours of the evening. Nevertheless, they proved to be agile when disturbed, often running along the branches or even jumping between twigs. The tail was kept low most of the time and appeared to be semi-prehensile. Based on our observations they retreat to safety inside the hollow branches of *Senegalia mellifera detinens* during the day, and emerge soon after dark to forage, mostly on thin branches but not restricted to the acacias. They likely avoid the ground and no specimen was found walking on soil or rock, but their agility allows them to move across neighboring trees, particularly where there is a high density of bushes, as at Maconjo, or where canopy contact is facilitated by uneven and steep slopes as in Sta Maria. A nocturnal arboreal foraging behavior has also

been recorded for another Angolan endemic gecko, the possibly closely related species *Kolekanos plumicaudus*, although the latter retreats into rock crevices for shelter (Agarwal et al. 2017).
The geckos probably prey on small insects found on the outer branches of bushes, and it is possible that they also feed on alates of the same termites that provide them with the sheltering habitat, although this could not be confirmed as gut content analyses were not performed. The vast hollow branches with narrow entrances likely preclude many snakes from preying on adults and eggs. In any case, one specimen of *Psammophis leopardinus* was collected, while actively moving at night among the outer branches of a blackthorn bush at Maconjo (Fig. 6A), strongly suggesting that it was hunting *A. ansorgii*.
At both localities the gecko *Hemidactylus* cf.

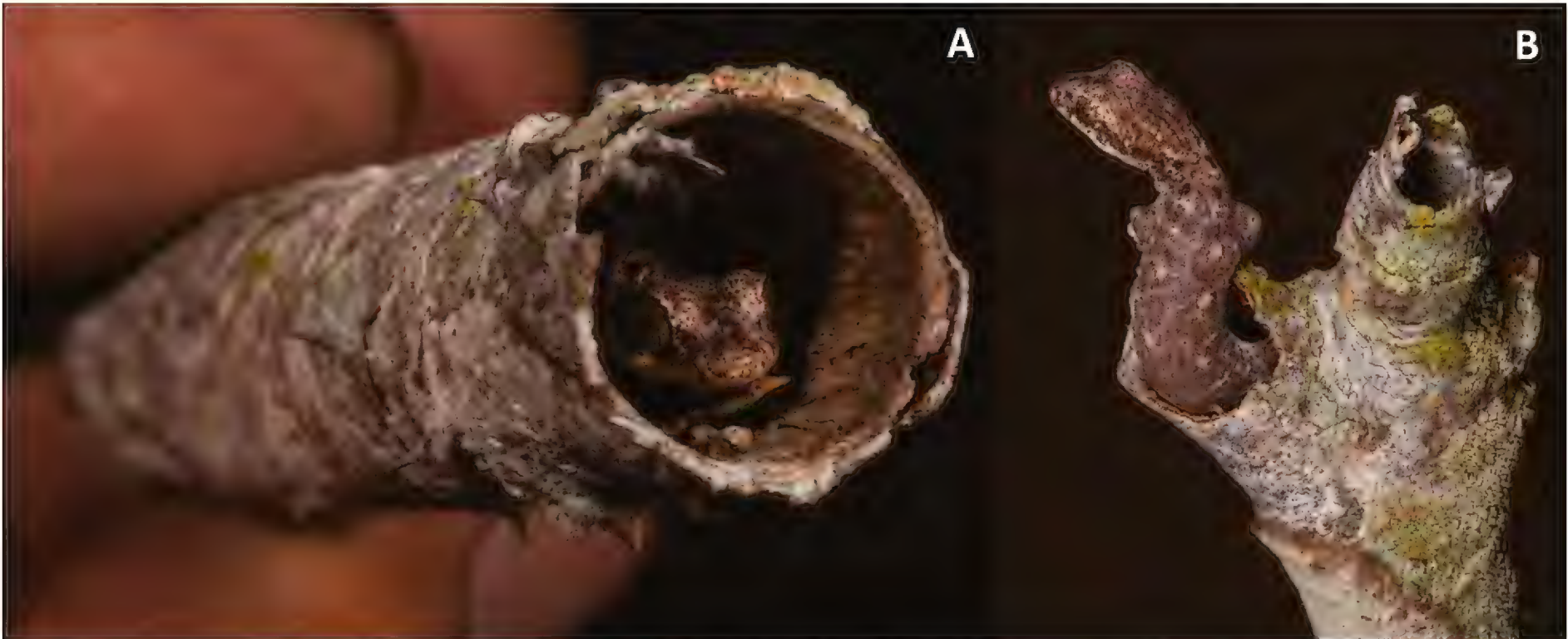


Fig. 3. *Afrogecko ansorgii* in its biotope, the termite-hollowed, thin branches of *Senegalia mellifera detinens*. (A) Coming out of a hollow blackthorn twig. (B) Specimen caught trapped inside a blackthorn branch.

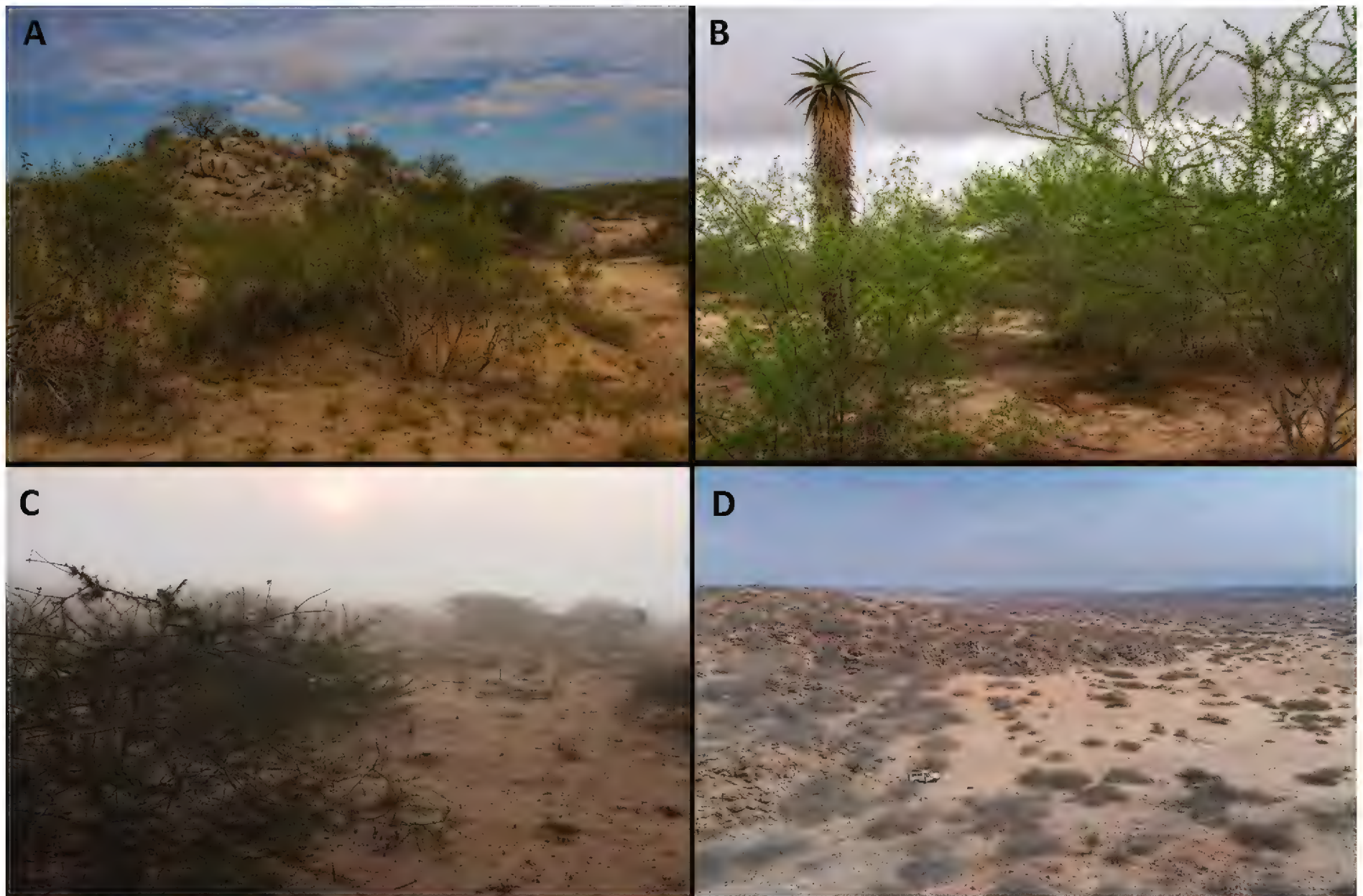


Fig. 4. Habitat of *Afrogecko ansorgii*. **(A)** Isolated granite outcrop beside the dry Cocumba stream near its confluence with Conjo Stream (12°52'S, 13°21'E, 355 m asl), the correct site of Boulenger's type locality 'Maconjo' for *Phyllodactylus ansorgii* and *Mabouia laevis*. **(B)** Spiny savanna of *Senegalia mellifera detinens* with *Terminalia prunioides* and *Aloe littoralis* at the type locality. **(C)** Scattered blackthorn bushes subject to thick morning fog and covered with lichen within which *Afrogecko ansorgii* was rediscovered. **(D)** Aerial view of dry spiny savannah at the head of a valley leading down to Cape Sta Maria, Benguela Province (13°31'S, 12°38'E, 288 m asl).

longicephalus was collected in syntopy in the same bushes, although usually foraging on thicker branches and closer to the ground. Other rupicolous reptile species on the surrounding rocks on both sites included *Pachydactylus caraculicus*, *Chondrodactylus fitzsimonsi*, *C. pullitzae*, *Trachylepis laevis* (Fig. 6B), *T. sulcata ansorgii*, *Agama planiceps*, the diurnal *Rhoptropus* cf. *benguellensis* and *R.* cf. *barnardi*; and the terrestrial species *Pachydactylus punctatus*, *Trachylepis acutilabris*, and *Pedioplanis benguellensis*. Species found in Sta Maria but not recorded at Maconjo were *Afroedura* cf. *bogerti*, *Pachydactylus* cf. *oreophilus*, *Cordylus namakuiyus*, and *Hemirhagerhis viperina*, while *Matobosaurus maltzahnii*, *Agama anchietae*, *Trachylepis binotata*, and *Psammophis leopardinus* were found at Maconjo but not at Sta Maria. These records include northern extension ranges of roughly 200 km or more for three gekkonids, *Chondrodactylus fitzsimonsi*, *Pachydactylus caraculicus*, and *Rhoptropus* cf. *barnardi*, and for the endemic and recently described *Cordylus namakuiyus* (Stanley et al. 2016).

Distribution range: So far, the gecko has only been found in the two referred localities of Maconjo and Cape Sta Maria, spanning across over 100 km on Benguela's coastal plain. It is likely that the species has a wider distribution range, where it may be locally common but

irregularly distributed, and dependent on very particular habitat requirements in association with *Senegalia mellifera detinens* and the kalotermitid termites.

New series and morphological variation: The new material obtained in both localities is consistent with the original description of *Afrogecko ansorgii*. The more slender form and the enlarged precloacal scales well distinguish this species from *A. porphyreus* (Daudin 1802), while the larger size, presence of femoral spurs and a slender semi-prehensile versus a broad flattened tail clearly separates it from the other Angolan endemic Leaf-toed Gecko *Kolekanos plumicaudus* (Haacke 2008). Boulenger's description, based on one of two available adult females, is terse but conforms to that of the era and is given below, while some measurements, including the new series, are presented in Table 1. Male specimens were obtained for the first time, allowing comparisons between sexes and localities. A detailed re-description of *A. ansorgii* is being prepared, which will also explore phylogenetic relationships and include a taxonomic review (data not shown). Original description of *Afrogecko ansorgii* (Boulenger 1907):

Head rather small, oviform, much longer than broad; snout not longer than the distance between the eye and the ear opening, which is small and oval. Body

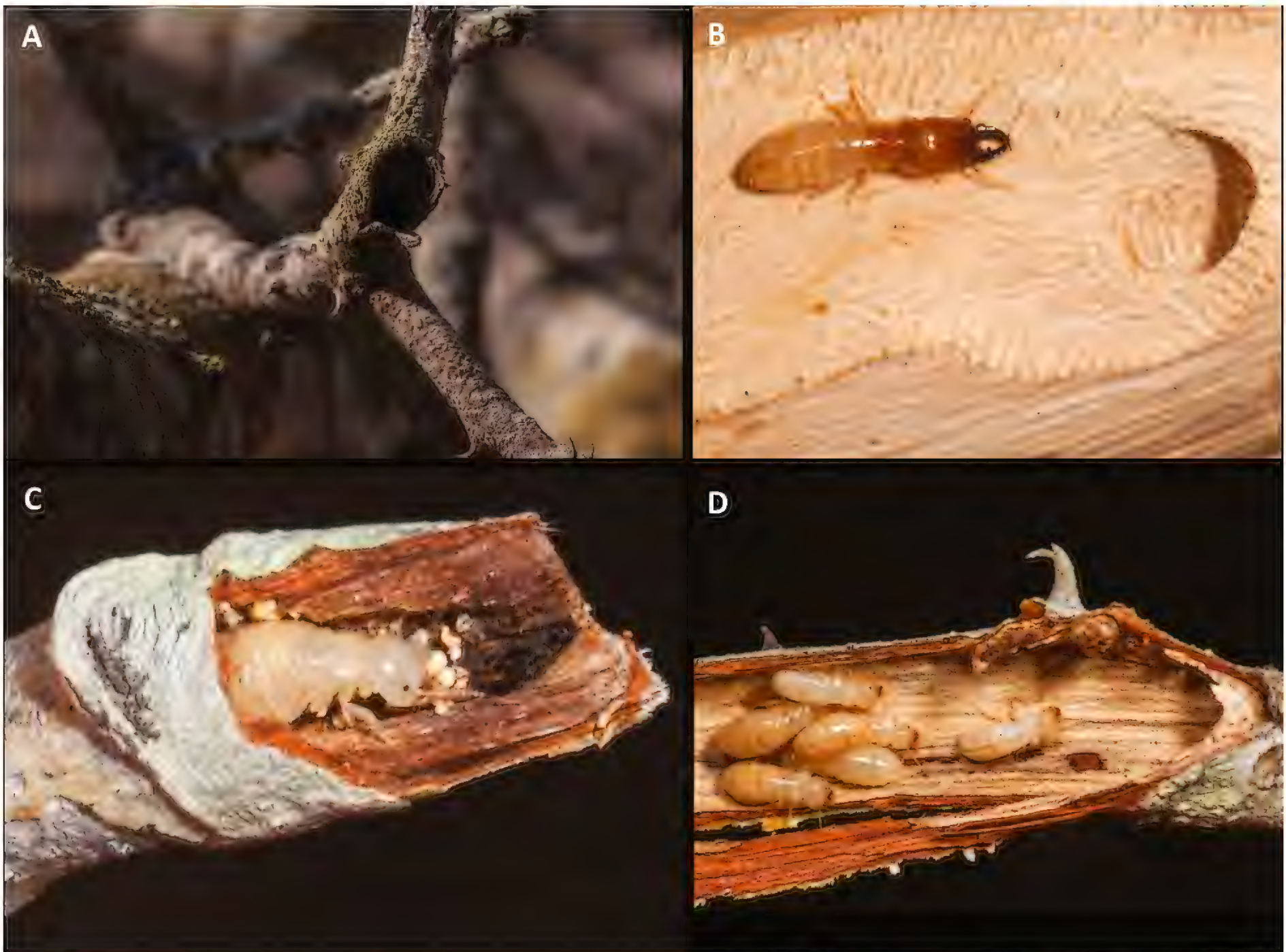


Fig. 5. Kalotermitid termites active on blackthorn bushes at type locality. (A) Hollow branch in tree. (B) Termite soldier and evidence of wood excavation. (C) Termite and eggs inside branch. (D) Workers active inside hollow branch.

very elongate; limbs moderate. Digits moderately depressed, with large, subtrapezoid terminal expansions; eight lamellae under the fourth toe. Head and body covered with uniform, smooth, flattened granules, which are larger on the snout and on the belly. Rostral twice as broad as deep, without cleft above; symphysial small, a little longer than broad; ten upper and as many lower labials; rostral and first upper labial entering the nostril; no chin-shields. Tail cylindrical, tapering, covered with uniform, small, quadrangular, smooth scales. A curved transverse series of eight or nine enlarged praeanal scales (indicating praeanal pores in the male?). Pale greyish brown above, with a series of large whitish spots along each side of the back; a dark streak on each side of the head and neck, passing through the eye; upper lip and lower parts white, with small brown spots.

Two females collected in the type locality were gravid with two eggs (Fig. 7A). Male specimens are noticeably smaller than females in snout-vent and total length, with the difference being more pronounced at the type locality. As predicted by Boulenger, males have 7–9 precloacal pores (Fig. 7B), corresponding to the enlarged scales found on females (Fig. 7C). Of note are the differences found between the series obtained at Maconjo and Sta Maria. Specimens from Maconjo proved to be consistently larger in size and overall measurements,

with females from Sta. Maria of approximately similar size as males from Maconjo (Table 1). Also striking is the presence of more cloacal spurs in the series from the type locality. Although not mentioned in the type description, we found one cloacal spur in females from Maconjo (Fig. 7A), and two spurs in males from same locality (Fig. 7B). On the other hand, females from Sta Maria showed no cloacal spurs, while at the same site all males but one had one single spur (Table 1).

Discussion

The traditional reliance of gekkonid systematics on digital morphology is often reflected in the generic names, with the Leaf-toed Geckos, *Phyllodactylus* being one of numerous examples. Until the end of the 20th century, gecko species assigned to *Phyllodactylus* had a patchy distribution, spread across five continents. Increasing awareness of both the antiquity of gekkotan lineages (Kluge 1987; Conrad and Norell 2007), of the taxonomic confusion generated by their conflicting conservative and sometimes convergent morphologies (Bauer et al. 1997; Gamble et al. 2012), and of the development and application of molecular phylogenetic studies (Han et al. 2004; Wiens et al. 2012), have resulted in a true understanding of gecko antiquity and diversity. Taxonomic re-arrangements to reflect this evolution and

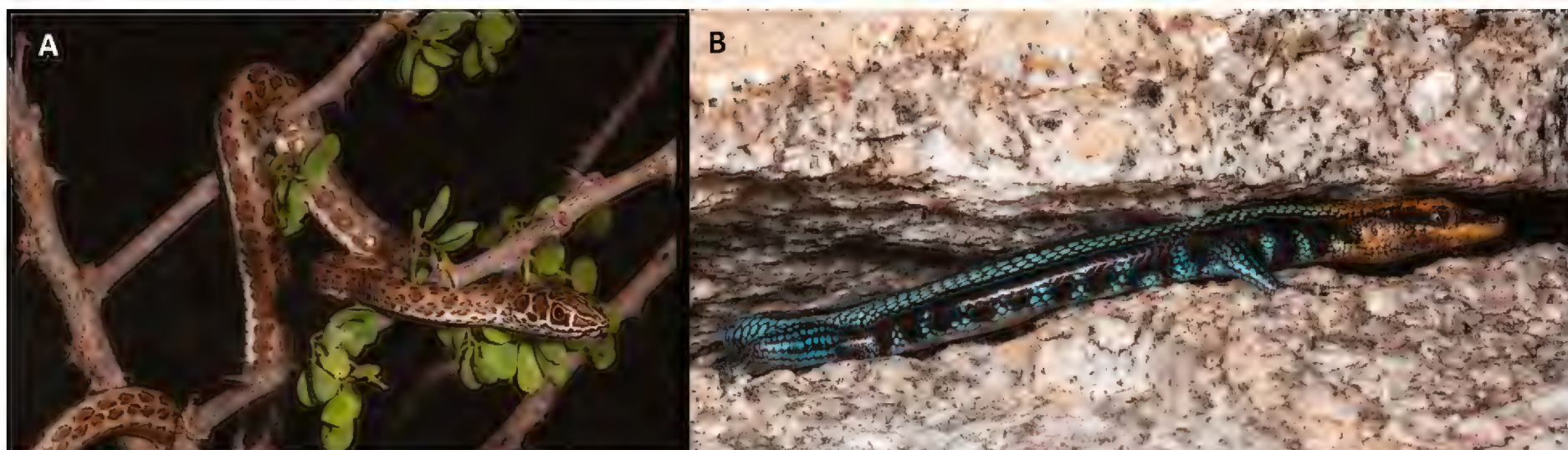


Fig. 6. (A) Specimen of *Psammophis leopardinus* foraging at night on a blackthorn bush at Maconjo, possibly preying on *A. ansorgii*. (B) First topotypic material of *Trachylepis laevis* collected in over 110 years, FKH0018 in life; note flattened body adapted to shelter among tight rocky spaces.

cryptic diversity has resulted in the genus *Phyllodactylus*, as understood for over 100 years, now being distributed among the families Diplodactylidae, Gekkonidae, and Phyllodactylidae and in at least 15 genera! It is thus not surprising that the generic affinities of *Afrogecko ansorgii* remain problematic. When initiating a major generic revision of phyllodactyline geckos, Bauer et al. (1997) provisionally assigned *ansorgii* (then known only from the types) to the genus *Afrogecko*, but noted that its affinities would need re-assessment on discovery of new material.

Subsequently Haacke (2008) described the new plume-tailed gecko from the Angolan Namib region that was again tentatively assigned as *Afrogecko plumicaudus* in the absence of genetic studies. The collection of additional new material by Wulf Haacke allowed its phylogenetic affinities to be assessed, whereupon it was transferred to a new gekkonine genus *Kolekanos* (Heinicke et al. 2014). Although all the original material was collected under thin, exfoliating rock slabs, the species was later shown to forage arboreally (Agarwal et al. 2017). In its (at least) partial arboreal behavior, *K. plumicaudus* is similar to *A. ansorgii*. Nevertheless, *A. ansorgii* is unique among southern African leaf-toed geckos, in being exclusively arboreal and by what appear to be some remarkable ecological adaptations. The morphological differences found in specimens obtained at the type locality and Sta Maria, namely in body size and number of cloacal spurs, likely reflect local adaptations and may be climatically driven. Further detailed morphological and genetic studies are ongoing to confirm the generic placement of *A. ansorgii* and explore intraspecific variation (data not shown).

Southwestern coastal Angola is subject to frequent advective fogs and relatively cool temperatures, caused by the cold offshore Benguela current, and experiences a climatic gradient of increased rainfall from the coast inland (Huntley 2019). The importance of advective fog and low clouds in shaping the local arid ecosystems has been relatively well studied in Namibia, where it has been estimated to represent up to five times the amount of precipitation provided by rain (Seeley and Henschel 1998). The frequency of this type of fog is high in coastal areas, but studies in the central Namib found the amount of precipitation to be highest around 35–60 km inland and below 500 m asl, where low strata clouds were

intercepted by local elevation (Lancaster 1984). Satellite imagery show that advective fog and low clouds penetrate further inland in the southern and northern sections of the Namib (Cermak 2012), but relatively little effort has focused on the role of fog and low clouds in Angola. Nevertheless, it has been suggested that the incidence of winter fog and low strata clouds is especially pronounced between the towns of Namibe and Benguela, leading to heavy morning dew and allowing for a local abundance of epiphytic lichens (Huntley 2019). The presence of these epiphytic lichens was recorded at the site of Sta Maria, where they often almost covered the branches of blackthorn bushes. Being close to the sea shore and situated in an extensive dry valley, the collecting site at Sta Maria is subject to frequent morning coastal

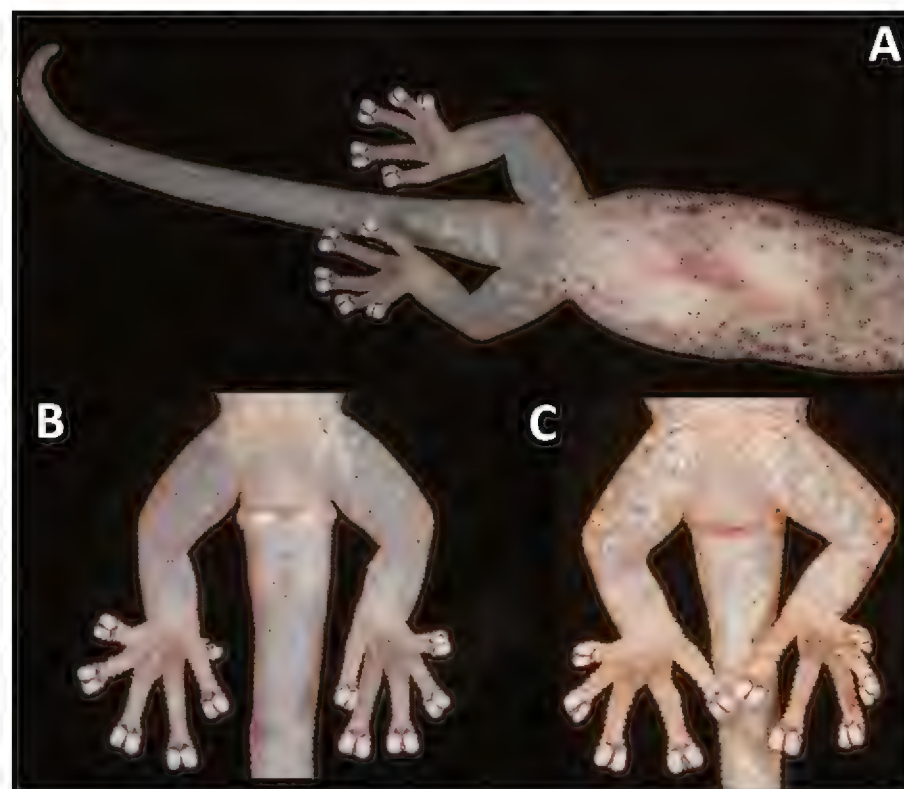


Fig. 7. (A) Female *Afrogecko ansorgii* from Maconjo, FKH0007, gravid with two eggs. Note enlarged precloacal scales and presence of one cloacal spur. (B) Cloacal region of male *A. ansorgii*, FKH0008. Note single chevron row of nine precloacal pores, hemipenial bulges (as paired light swellings) and cloacal spurs (small white tubercular scales on lateral surfaces of tail base) in the male. (C) Cloacal region of female *A. ansorgii*, FKH0009. Note lack of precloacal pores on the chevron row of enlarged scales or of enlarged tubercular lateral scales on tail base. Both sexes have delicate elongate toes with a single pair of terminal scansors. The cylindrical tail is finely scaled, and non-verticillate, lacking obvious lateral constrictions.

advective fogs which become trapped among high rocky slopes. Although lichens were less abundant at Maconjo, the type locality also appeared to be regularly subject to thick foggy conditions. Situated at around 40 km from the coast and 400 m asl, Maconjo lies at the base of a north-south oriented orographic step, characterized by mountainous granitic hills that elevate the coastal plateau further east to above 800 m asl, and therefore topography likely plays a role in containing fog and low strata clouds and, ultimately, shaping the ecological conditions of local spiny savannas. The blackthorn bush holds a reputation for being termite-resistant (Schmidt et al. 2002). Nevertheless, in both Maconjo and Sta Maria we found ‘drywood’ termites (family Kalotermitidae) to be present inside dead blackthorn branches, often leaving them neatly excavated. This was especially evident at the drier environment of Sta Maria, where large numbers of dead branches break and accumulate on the ground underneath the bush canopy, thus providing an abundance of sheltering habitat for the gecko. Drywood termites are well adapted to arid ecosystems but may also require some moisture and tend to be uncommon or patchily distributed. It appears therefore that the local distribution of termites is driven by specific environmental conditions affecting the bushes of *Senegalia mellifera detinens*, like altitude and rainfall, atmospheric moisture provided by fog or low clouds, and the frequency and severity of droughts. In regions with relatively higher moisture from average rainfall, these blackthorn savannas may not accumulate enough dead wood and the conditions might be unsuitable for the termites and ultimately the gecko. On the other hand, under extremely dry conditions the termites may not thrive, or the density of bushes might be too low, not providing enough shelter or foraging habitat for this exclusively arboreal gecko. Therefore, the dry coastal blackthorn savannas that experience low rainfall but are subject to frequent fogs, seem to constitute the ‘sweet spot’ habitat for the species.

Formerly known locally as *Deserto de Mossamedes*, the northernmost extension of the Namib Desert stretches north along the Atlantic coast of Angola from the Angola-Namibia border for 450 km to about the city of Benguela. Fronting the Atlantic Ocean to the west, it gradually ascends in elevation eastward to a semiarid plain dominated by acacia and mopane [African ironwood] trees that abuts the steep Serra de Chela escarpment. Characterized by gravel plains and rock platforms interspersed with sand dune fields, the Angolan Namib is part of a broader ecoregion defined as Kaokoveld Desert (Burgess 2004). It is believed that ancient climatic stability, a mosaic of substrates and incidence of coastal fogs contribute to high species richness and rates of endemism in the Namib region (Seeley et al. 1998), and the Kaokoveld is often referred to as a regional center of endemism for flora (Van Wyk and Smith 2011), beetles (Koch 1961), and lizards (Lewin et al. 2016; Branch et al. 2019b). Nevertheless, the boundaries of the northern Kaokoveld have remained poorly defined and although Burgess (2004) depicted the ecoregion along the Angolan coast to about Benguela town, other authors have set the northern limit at Lucira, in Namibe Province (e.g., Craven 2009; Branch et al. 2019). Between Lucira

and Benguela the semi-arid ecosystems characterized by a diversity of succulent plants progressively gives way to semi-arid dry savannas dominated by acacia species (Barbosa 1970), thus placing our surveyed sites on the fringes of the Kaokoveld ecoregion. Historical and recent herpetological work on the Angolan arid ecosystems has mostly focused in the southernmost regions of the Angolan Kaokoveld (Marques et al. 2018; Branch et al. 2019b), and about one-third of national reptile diversity is known to occur in the Namibe Province alone, with gekkonids being the most speciose group (Ceriaco et al. 2016). In comparison, less effort has been directed to Benguela Province, yet the diversity and new extension ranges here reported suggest that the herpetological richness of the province may have been underestimated. It is likely that future surveys across the northern limits of the Kaokoveld will further increase the regional lists, unveil cryptic diversity, and underline the ecological significance of the local spiny semi-arid savannas associated with fog and low strata clouds. Apparently restricted to Benguela’s Kaokoveld and strongly associated with the local fog ecosystem, the now rediscovered Ansorge’s gecko remains as one of the most unique and iconic representatives of Angolan herpetofauna.

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Literature Cited

- Agarwal I, Ceriaco L, Bauer AM, Bandeira S. 2017. *Kolekanos plumicaudus* (Slender Feathered-tailed Gecko): Habitat use. *Herpetological Review* 48(3): 649–650.
- Barbosa LAG. 1970. *Carta Fitogeográfica de Angola*. Instituto de Investigação Científica de Angola, Luanda, Angola. 323 p.
- Bauer AM, Branch WR, Haacke WD. 1993. The herpetofauna of the Kamanjab area and adjacent Damaraland, Namibia. *Madoqua* 18(2): 117–145.
- Bauer AM, Good DA, Branch WR. 1997. The taxonomy of the Southern African leaf-toed geckos (Squamata: Gekkonidae), with a review of Old World “*Phyllodactylus*” and the description of five new genera. *Proceedings of the California Academy of Sciences* 49: 47–497.
- Bocage JVB. 1895. *Herpétologie d’Angola et du Congo*. Imprimerie Nationale, Lisbonne, Portugal. 203 p.

- Boulenger GA. 1905. A list of the batrachians and reptiles collected by Dr. W. J. Ansorge in Angola, with descriptions of new species. *Annals and Magazine of Natural History, Series 7* 16: 105–115.
- Boulenger GA. 1907. Descriptions of three new lizards and a new frog, discovered by Dr. W. J. Ansorge in Angola. *Annals and Magazine of Natural History, Series 7* 19: 212–214.
- Branch WR. 2016. Preface, *Amphibian & Reptile Conservation* Special Angola and Africa Issue. *Amphibian & Reptile Conservation* 10(2) [Special Section]: 2, i–iii (e128).
- Branch WR. 2018. Snakes of Angola: an annotated checklist. *Amphibian & Reptile Conservation* 12(2) [General Section]: 41–82 (e159).
- Branch WR, McCartney CJ. 1992. A report on a small collection of reptiles from southern Angola. *Journal of the Herpetological Association of Africa* 41: 1–3.
- Branch WR, Baptista N, Vaz Pinto P. 2018. Angolan Amphisbaenians: rediscovery of *Monopeltis luandae* Gans 1976, with comments on the type locality of *Monopeltis perplexus* Gans 1976 (Sauria: Amphisbaenidae). *Herpetological Notes* 11: 603–606.
- Branch WR, Baptista N, Keates C, Edwards S. 2019a. Rediscovery, taxonomic status and phylogenetic relationships of two rare and endemic snakes (Serpentes: Psammophinae) from the southwestern Angolan plateau. *Zootaxa* 4590: 342–366.
- Branch WR, Vaz Pinto P, Baptista N, Conradie W. 2019b. The reptiles of Angola: history, diversity, endemism and hotspots. Pp. 283–334 In: *Biodiversity of Angola. Science & Conservation: A Modern Synthesis*. Editors, Huntley BJ, Russo V, Lages F, Ferrand N. Springer Nature, Cham, Switzerland. 549 p.
- Broadley DG. 1980. A revision of the African snake genus *Prosymna* Gray (Colubridae). *Occasional Papers of the National Museums and Monuments of Rhodesia* 6(7): 481–556.
- Broadley DG, Hughes B. 2000. A revision of the African genus *Hemirhagerrhis* Boettger 1893 (Serpentes: Colubridae). *Syntarsus* 6: 1–17.
- Burgess N, Hales JA, Underwood E, Dinerstein E, Olson D, Itoua I, Schipper J, Ricketts T, Newman K. 2004. *Terrestrial Ecoregions of Africa and Madagascar: A Conservation Assessment*. Island Press, Washington, DC, USA. 501 p.
- Ceríaco LMP, de Sá SAC, Bandeira S, Valério H, Stanley EL, Kuhn AL, Marques MP, Vindum JV, Blackburn DC, Bauer AM. 2016. Herpetological survey of Iona National Park and Namibe Regional Natural Park, with a synoptic list of the amphibians and reptiles of Namibe Province, Southwestern Angola. *Proceedings of the California Academy of Sciences* 63(2): 15–61.
- Ceríaco LMP, de Sá SAC, Marques MP, Bandeira S, Agarwal I, Stanley EL, Bauer AM, Heinicke MP, Blackburn DC. 2018. A new earless species of *Poyntonophrynus* (Anura, Bufonidae) from the Serra da Neve Inselberg, Namibe Province, Angola. *ZooKeys* 780: 109–136.
- Cermak J. 2012. Low clouds and fog along the South-Western African coast. Satellite-based retrieval and spatial patterns. *Atmospheric Research* 116: 15–21.
- Chapin JP. Undated. Manuscript: William J. Ansorge, summary of itineraries in Angola, 1903–1910, *American Geographical Society of New York Records*, 1723–2010, bulk 1854–2000.
- Conrad JL, Norell MA. 2006. High-resolution x-ray computed tomography of an Early Cretaceous gekkonomorph (Squamata) from Öösh (Övörkhanga; Mongolia). *Historical Biology* 18(4): 405–431.
- Conradie W, Branch WR, Measey JG, Tolley KA. 2012a. A new species of *Hyperolius* Rapp, 1842 (Anura: Hyperoliidae) from the Serra da Chela mountains, south-western Angola. *Zootaxa* 3269: 1–17.
- Conradie W, Measey JG, Branch WR, Tolley KA. 2012b. Revised phylogeny of African sand lizards (*Pedioplanis*), with the description of two new species from south-eastern Angola. *African Journal of Herpetology* 61(2): 91–112.
- Conradie W, Branch WR, Tolley KA. 2013. Fifty shades of grey: giving colour to the poorly known Angolan Ashy Reed Frog (Hyperoliidae: *Hyperolius cinereus*), with the description of a new species. *Zootaxa* 3635(3): 201–223.
- Conradie W, Bills R, Branch WR. 2016. The herpetofauna of the Cubango, Cuito and lower Cuando river catchments of south-eastern Angola. *Amphibian & Reptile Conservation* 10(2) [Special Section]: 6–36 (e126).
- Craven P. 2009. Phytogeographic study of the Kaokoveld centre of endemism. Ph.D. Dissertation, University of Stellenbosch, Stellenbosch, South Africa. 234 p.
- Crawford-Cabral J, Mesquitela LM. 1989. *Índice Toponímico de Colheitas Zoológicas em Angola*. Instituto de Investigação Científica Tropical, Centro de Zoologia, Lisbon, Portugal. 206 p.
- de Andrade AAB. 1985. *O Naturalista José de Anchieta*. Estudos de História e Cartografia Antiga, Memórias 24. Instituto de Investigação Científica Tropical, Centro de Estudos de História e Cartografia Antiga, Lisboa, Portugal. 187 p.
- Ernst R, Nianguesso ABT, Lautenschläger T, Barej MF, Schmitz A, Hölting M. 2014. Relicts of a forested past: southernmost distribution of the hairy frog genus *Trichobatrachus* Boulenger, 1900 (Anura: Arthroleptidae) in the Serra do Pingano region of Angola with comments on its taxonomic status. *Zootaxa* 3779(2): 297–300.
- FitzSimons VF. 1959. Some new reptiles from southern Africa and southern Angola. *Annals of the Transvaal Museum* 23: 405–409.
- Gamble T, Greenbaum E, Jackman TR, Russell AP, Bauer AM. 2012. Repeated origin and loss of adhesive toepads in geckos. *PLoS One* 7(6): e39429.
- Han D, Zhou K, Bauer AM. 2004. Phylogenetic relationships among gekkotan lizards inferred from C-mos nuclear DNA sequences and a new classification of the Gekkota. *Biological Journal of the Linnean Society* 83(3): 353–368.
- Heinicke MP, Daza JD, Greenbaum E, Jackman TF, Bauer AM. 2014. Phylogeny, taxonomy and biogeography of a circum-Indian Ocean clade of leaf-toed geckos (Reptilia: Gekkota), with a description of two new genera. *Systematics and Biodiversity* 12(1): 23–42.

- Hellmich W. 1957. Herpetologische Ergebnisse einer Forschungsreise in Angola. *Veröffentlichungen der Zoologischen Staatssammlung München* 5: 1–92.
- Huntley BJ. 2009. SANBI/ISCED/UAN *Angolan Biodiversity Assessment Capacity Building Project. Report on Pilot Project*. Unpublished report to Ministry of Environment, Luanda, Angola. 97 p.
- Huntley BJ. 2019. Angola in outline: physiography, climate and patterns of biodiversity. Pp. 15–42 In: *Biodiversity of Angola. Science & Conservation: A Modern Synthesis*. Editors, Huntley BJ, Russo V, Lages F, Ferrand N. Springer Nature, Cham, Switzerland. 549 p.
- Huntley BJ, Ferrand N. 2019. Angolan biodiversity: towards a modern synthesis. Pp. 3–14 In: *Biodiversity of Angola. Science & Conservation: A Modern Synthesis*. Editors, Huntley BJ, Russo V, Lages F, Ferrand N. Springer Nature, Cham, Switzerland. 549 p.
- Huntley BJ, Francisco P. 2015. *Relatório sobre a Expedição Avaliação rápida da Biodiversidade da região da Lagoa Carumbo, Lunda-Norte – Angola/ Biodiversity Rapid Assessment of the Carumbo Lagoon Area, Lunda-Norte*. Angola Ministerio do Ambiente, Luanda, Angola. 219 p.
- Kluge AG. 1987. Cladistic relationships in the Gekkonoidea (Squamata, Sauria). *Miscellaneous Publications Museum of Zoology, University of Michigan* 173: 1–54.
- Koch C. 1961. Some aspects of abundant life in the vegetation-less sand at the Namib Desert dunes. *Scientific Papers of the Namib Desert Research Station* 1961(1): 9–92.
- Lancaster J, Lancaster N, Seely MK. 1984. Climate of the central Namib Desert. *Madoqua* 14(1): 5–61.
- Laurent RF. 1964. Reptiles et amphibiens de l'Angola (Troisième contribution). *Publicações Culturais da Companhia de Diamantes de Angola* 67: 11–165.
- Lewin A, Feldman A, Bauer AM, Belmaker J, Broadley DG, Chirio L, Itescu Y, LeBreton M, Maza E, Mierte D, et al. 2016. Patterns of species richness, endemism and environmental gradients of African reptiles. *Journal of Biogeography* 43(12): 2,380–2,390.
- Loveridge A. 1944. New geckos of the genera *Afroedura*, new genus, and *Pachydactylus* from Angola. *American Museum Novitates* 1254: 1–4.
- Loveridge A. 1947. Revision of the African lizards of the family Gekkonidae. *Bulletin of the Museum of Comparative Zoology* 98: 1–469, Plates 1–7.
- Marques MP, Ceriaco LMP, Blackburn DC, Bauer AM. 2018. Diversity and distribution of the amphibians and terrestrial reptiles of Angola. Atlas of historical and bibliographic records (1840–2017). *Proceedings of the California Academy of Sciences* 65(Supplement II): 1–501.
- Monard A. 1937. Contribution à l'herpétologie d'Angola. *Arquivos do Museu Bocage* 8: 19–154.
- Paluh DJ, Bauer AM. 2017. Comparative skull anatomy of terrestrial and crevice-dwelling *Trachylepis* skinks (Squamata: Scincidae) with a survey of resources in scincid cranial osteology. *PLoS One* 12(9): e0184414.
- Schmidt E, Lotter M, McClelland W. 2002. *Trees and Shrubs of Mpumalanga and Kruger National Park*. Jacana Media, Johannesburg, South Africa. 702 p.
- Seely M, Henschel JR. 1998. The climatology of Namib fog. Pp. 353–356 In: *Proceedings of the First International Conference on Fog and Fog Collection*. Editors, Schemenauer RS, Bridgman H. Conference on Fog and Fog Collection, North York, Ontario, Canada. 492 p.
- Seely M, Henschel J, Robertson M. 1998. The ecology of fog in Namib Desert dunes. Pp. 183–186 In: *Proceedings of the First International Conference on Fog and Fog Collection*. Editors, Schemenauer RS, Bridgman H. Conference on Fog and Fog Collection, North York, Ontario, Canada. 492 p.
- Stanley EL, Ceriaco, LMP, Bandeira S, Valerio H, Bates MF, Branch WR. 2016. A review of *Cordylus machadoi* (Squamata: Cordylidae) in southwestern Angola, with the description of a new species from the Pro-Namib desert. *Zootaxa* 4061(3): 201–226.
- Steyn W, Mitchell JL. 1965. A new scincid genus and a new record from South West Africa. *Cimbebasia* 12: 2–12.
- Uetz P, Freed P, Hošek J. 2019. The Reptile Database. Available: <http://www.reptile-database.org> [Accessed: 1 March 2019].
- Van Wyk AE, Smith GF. 2001. *Regions of Floristic Endemism in Southern Africa: A Review with Emphasis on Succulents*. Umdaus Press, Hatfield, South Africa. 199 p.
- Wiens JJ, Hutter CR, Mulcahy DG, Noonan BP, Townsend TM, Sites Jr JW, Reeder TW. 2012. Resolving the phylogeny of lizards and snakes (Squamata) with extensive sampling of genes and species. *Biology Letters* 8(6): 1,043–1,046.



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Bill Branch (William R. Branch) was born in London, United Kingdom. He was employed as Curator of Herpetology at the Port Elizabeth Museum for over 30 years (1979–2011), and upon his retirement he was appointed Curator Emeritus Herpetology until his death in October 2018. Bill's herpetological studies concentrated mainly on the systematics, phylogenetic relationships, and conservation of African reptiles, but he has been involved in numerous other studies on the reproduction and diet of African snakes. He has published over 300 scientific articles, as well as numerous popular articles and books. The latter include: *South African Red Data Book of Reptiles and Amphibians* (1988), *Dangerous Snakes of Africa* (1995, with Steve Spawls), *Field Guide to the Reptiles of Southern Africa* (1998), *Tortoises, Terrapins and Turtles of Africa* (2008), and *Atlas and Red Data Book of the Reptiles of South Africa, Lesotho and Swaziland* (multi-authored, 2014), as well as smaller photographic guides. In 2004, he was the 4th recipient of the "Exceptional Contribution to Herpetology" award of the Herpetological Association of Africa. Bill has undertaken field work in over 16 African countries, and described nearly 50 species, including geckos, lacertids, chameleons, cordylids, tortoises, adders, and frogs.





Phylogeography of the East African Serrated Hinged Terrapin *Pelusios sinuatus* (Smith, 1838) and resurrection of *Sternothaerus bottegi* Boulenger, 1895 as a subspecies of *P. sinuatus*

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Abstract.—*Pelusios sinuatus* is distributed in East Africa from southern Ethiopia and Somalia to northeastern South Africa. Inland it reaches westernmost Zimbabwe, Rwanda, and Burundi. Despite this wide range, which spans in north-south direction across 3,500 km and in east-west direction more than 1,500 km, no geographic variation has been described. However, using phylogenetic and haplotype network analyses of mitochondrial and nuclear DNA (2,180 bp and 2,132 bp, respectively), phylogeographic variation is herein described, with two distinct genealogical lineages. One occurs in the northern and central parts of the distribution range, and the other is in the south. Terrapins representing the southern lineage attain a smaller maximum body size than terrapins from the northern and central parts of the range. The distribution ranges of the two lineages abut in the border region of Botswana, South Africa, and Zimbabwe. We conclude that each lineage represents a distinct subspecies, with the nominotypical subspecies *Pelusios sinuatus sinuatus* (Smith, 1838) occurring in the south and the newly recognized subspecies *Pelusios sinuatus bottegi* (Boulenger, 1895) in the central and northern distribution range. We found phylogeographic structuring within each subspecies and propose that the differentiated population clusters should be recognized as Management Units.

Keywords. management units, Pelomedusidae, systematics, taxonomy, Testudines, turtle

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Introduction

The freshwater turtle genus *Pelusios* comprises 17 species distributed across sub-Saharan Africa, with most likely introduced populations on Madagascar, the Seychelles, and Guadeloupe in the Lesser Antilles (Fritz et al. 2011, 2013; Stuckas et al. 2013; TTWG 2017). All species of *Pelusios* are side-necked turtles characterized by a plastral hinge that allows partial or complete closure of the anterior shell opening (Bramble and Hutchison 1981; Branch 2008). Together, *Pelusios* and its African-Arabian sister genus *Pelomedusa*, which has 10 formally recognized species (Petzold et al. 2014; TTWG 2017), constitute the family Pelomedusidae that is sister to the New World-Madagascan family Podocnemididae.

Pelomedusids and podocnemidids are both of Gondwanan origin (de Broin 1988; Noonan 2000) and, together with the Australasian and South American family Chelidae, represent the chelonian suborder Pleurodira (side-necked turtles; TTWG 2017).

In addition to a number of species with localized narrow distribution, *Pelusios* also includes species with wide distributions (Branch 2008; TTWG 2017). The Serrated Hinged Terrapin, *P. sinuatus* (Smith, 1838), has one of the largest distribution ranges of all *Pelusios* species. It occurs in East Africa from southern Ethiopia and Somalia southwards to Eswatini (formerly Swaziland) and northeastern South Africa (Branch 2008; TTWG 2017; Fig. 1). The Serrated Hinged Terrapin has the largest body size in its genus and may reach up to

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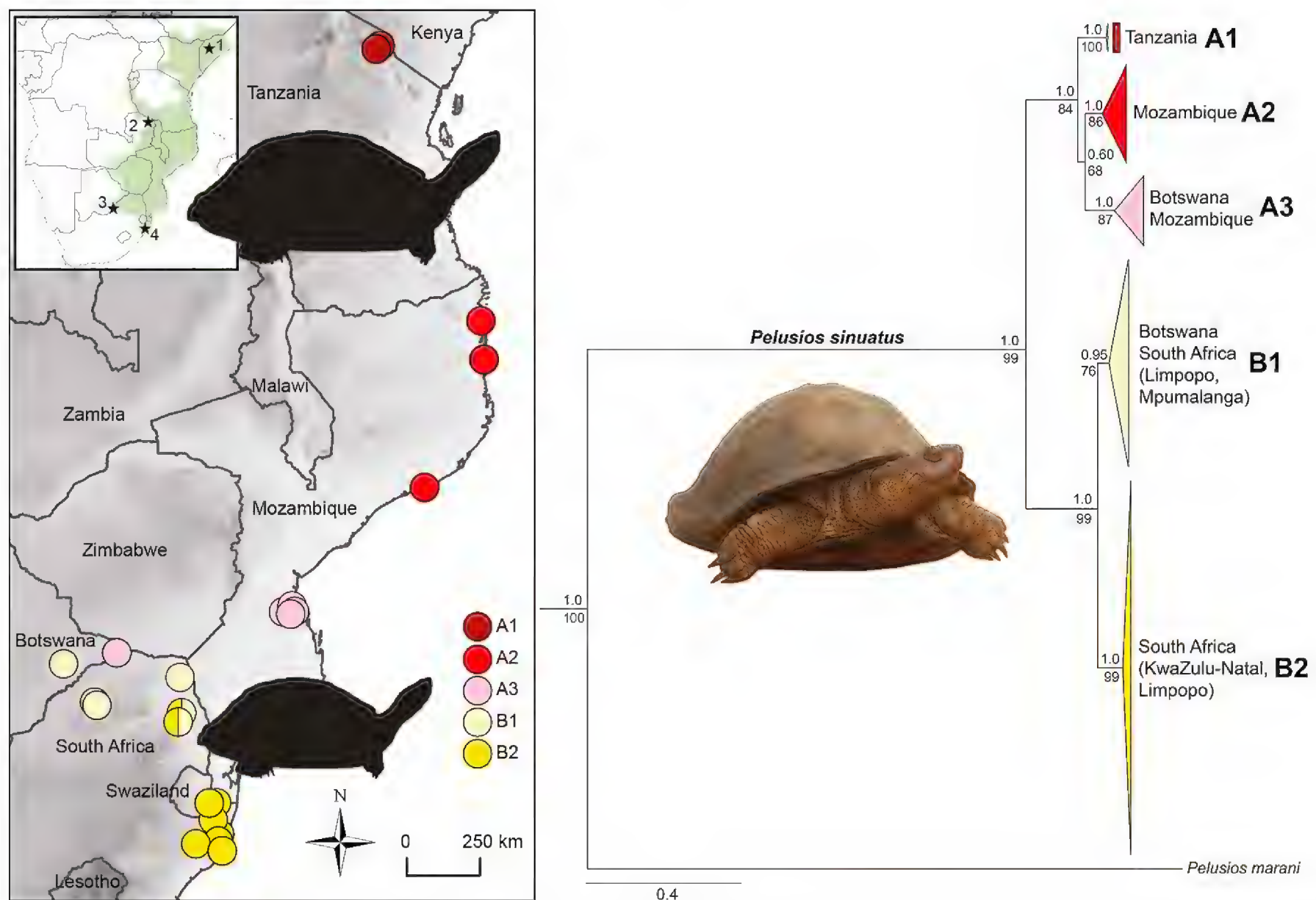


Fig. 1. Left: Sampling sites and mitochondrial identity of *Pelusios sinuatus* used in the present study. Divided symbols indicate syntopic occurrences of the respective subclades. Terrapin shapes symbolize differences in maximum sizes of the northern and southern *P. sinuatus* (see Discussion section). **Inset:** Distribution range of *P. sinuatus* according to the TTWG (2017) with the type localities of taxa referred to this species. (1) *Sternothaerus bottegi* Boulenger, 1895: Bardere (Bardera), Somalia; (2) *Pelusios sinuatus leptus* Hewitt, 1933: Isoka, Zambia; (3) *Sternotherus sinuatus* Smith, 1838 – restricted type locality (Broadley, 1981): confluence of Crocodile and Marico Rivers, Limpopo (Transvaal); (4) *Pelusios sinuatus zuluensis* Hewitt, 1927: Mzinene River (Umsinene River, Zululand), KwaZulu-Natal. **Right:** Bayesian tree for 61 *Pelusios sinuatus* using 2,180 bp of mitochondrial DNA. Clades are collapsed to cartoons showing the deepest genetic divergence within each clade. Outgroup *Pelomedusa variabilis* was removed for clarity. Numbers above the nodes are posterior probabilities; below the nodes, thorough bootstrap values under ML. Full trees are available from <https://figshare.com/s/52a7af23cff3aa08ea75>. **Inset:** Adult *Pelusios sinuatus*, Bonamanzi, KwaZulu-Natal, South Africa.

55 cm in shell length (Spawls et al. 2002). In contrast to other *Pelusios* species, *P. sinuatus* is a deep-water terrapin that occurs in perennial rivers, lakes, and larger man-made water bodies in savannah regions. During the rainy season, Serrated Hinged Terrapins move overland, and they colonize smaller water bodies, like pans and waterholes (Broadley and Boycott 2009). In eight other widespread *Pelusios* species, Fritz et al. (2013) and Kindler et al. (2016) found that phylogeographic patterns were not correlated with habitat type, with some species displaying pronounced phylogeographic structuring and others not. Among the studied savannah species, *P. rhodesianus* showed a deep phylogeographic structure and could actually represent a species complex (Kindler et al. 2016), whereas phylogeographic structuring in *P. nanus* and *P. subniger* was negligible. However, the westernmost studied population originally identified as *P. subniger* (in Democratic Republic of the Congo) was found to represent a genetically distinct undescribed species. Unfortunately, samples from the northern distribution area of *P. subniger* were not available for study, so nothing is known about the genetic identity of the northern populations (Fritz et al. 2013; Kindler et al. 2016). Another savannah species (*P. castanoides*), with

a distribution range similar to that of *P. sinuatus*, was characterized by moderate phylogeographic variation (Fritz et al. 2013).

Until now, neither the morphological nor the phylogeographic variation of *P. sinuatus* has been studied systematically. The species has traditionally been regarded as monotypic (Ernst and Barbour 1989; TTWG 2017; Wermuth and Mertens 1961, 1977), even though Hewitt (1927, 1933) had described two subspecies from South Africa and Zambia that were soon synonymized (Loveridge 1936). However, using mitochondrial and nuclear DNA sequences of only two samples from KwaZulu-Natal (South Africa) and another one from Botswana, Fritz et al. (2011) found two genetic lineages, suggesting that an in-depth investigation of genetic variation may reveal further differentiation. The present paper presents the first assessment of the genetic variation of *P. sinuatus* across its range. The results are discussed with respect to taxonomy and two subspecies are recognized within *P. sinuatus*. To this end, *Sternothaerus bottegi* Boulenger, 1895 is resurrected from the synonymy of *P. sinuatus* (Smith, 1838), in which it was placed soon after its description (Calabresi 1916; Siebenrock 1916).

Materials and Methods

Sampling, chosen loci, and general data evaluation strategy: Sixty-one samples of *Pelusios sinuatus* from Botswana, Mozambique, South Africa, and Tanzania were studied, including previously published data for three terrapins (Appendix 1). The same mitochondrial and nuclear DNA fragments were targeted as in earlier studies on *Pelusios* (Fritz et al. 2011; Kindler et al. 2016). Three mitochondrial DNA fragments were sequenced (12S, cyt *b*, and ND4 with adjacent DNA coding for tRNAs). In addition, two protein-coding nuclear genes (Cmos and Rag2) and intron 1 of the nuclear R35 gene were sequenced. Details of DNA isolation, PCR, and sequencing are described in Kindler et al. (2016). The 12S sequences obtained were up to 398 bp long (with gaps); the cyt *b* sequences were up to 913 bp; and the mtDNA sequences comprising the partial ND4 gene plus adjacent DNA coding for tRNAs were up to 869 bp long. All nuclear DNA blocks could be sequenced directly. Cmos sequences had lengths of up to 358 bp; R35 sequences, up to 1,101 bp; and Rag2 sequences, up to 673 bp. Sequences were aligned and inspected using BioEdit 7.0.5.2 (Hall 1999). All sequences aligned perfectly and gaps occurred only in sequence blocks not coding for proteins.

Mitochondrial DNA is maternally inherited, whereas nuclear loci are inherited biparentally. Moreover, mtDNA is prone to introgression, including across species borders, which often leads to conflicting results for the two marker systems (Currat et al. 2009; Funk and Omland 2003; Kehlmaier et al. 2019; Sloan et al. 2017; Toews and Brelsford 2012). To avoid the risk of such distortion, mitochondrial and nuclear sequence data were examined separately.

Phylogenetic analyses: Individual mtDNA fragments were concatenated for phylogenetic analyses, and this data set was combined with previously published sequences, resulting in an alignment of 2,180 bp length. The dataset included 61 sequences of *Pelusios sinuatus* and, as outgroups, one sequence each of *Pelomedusa variabilis* and *Pelusios marani*. European Nucleotide Archive (ENA) accession numbers and collection sites are given in Appendix 1. The best partitioning scheme was determined using PartitionFinder (Lanfear et al. 2012) and the Bayesian Information Criterion (BIC). Three partitioning schemes were tested: (1) unpartitioned, (2) partitioned by mtDNA fragment, and (3) partitioned by gene and codon position with DNA not coding for proteins (i.e., 12S and DNA coding for tRNAs) corresponding to one additional partition each. According to the results of PartitionFinder, scheme (3) was selected.

Phylogenetic relationships were inferred using Bayesian and Maximum Likelihood (ML) approaches. Bayesian trees were obtained with MrBayes 3.2.6 (Ronquist et al. 2012) using the partitioning scheme and evolutionary models shown in Table 1 and default parameters. Two parallel runs, each with four chains, were conducted. The chains ran for 10 million generations, with every 500th generation sampled. The calculation parameters were analyzed using a burn-in of 2.5

million generations to assure that both runs converged. Subsequently, only the plateau of the remaining trees was sampled using the same burn-in, and a 50% majority rule consensus tree was generated. Tracer 1.7 (Rambaut et al. 2018) served to check for convergence of the runs using the Effective Sample Sizes (ESS) of parameters, and resulted in ESSs over 200 after discarding the burn-in. In addition, phylogenetic relationships were inferred under ML using RAxML 7.2.8 (Stamatakis 2006) and the GTR+G substitution model across all partitions. Five independent ML searches were performed using different starting conditions and the fast bootstrap algorithm to explore the robustness of the results by comparing the best trees. Then, 1,000 non-parametric thorough bootstrap replicates were calculated and the values were plotted against the best tree.

Parsimony networks: For each mitochondrial and nuclear DNA fragment, a parsimony network was constructed using Popart (<http://popart.otago.ac.nz>). Since the underlying TCS algorithm is sensitive to missing data, a few individuals represented by short sequences were excluded. In addition, for achieving complete coverage, the lengths of mtDNA sequences were trimmed, resulting in an alignment of 348 bp length for 12S, 784 bp for cyt *b*, and 737 bp for ND4 + DNA coding for tRNA. For network construction of nuclear data, heterozygous sequences of R35 were phased using the Phase algorithm in DnaSP 5.10 (Librado and Rozas 2009), and two identical copies for homozygous sequences of all loci were included. Nuclear DNA sequences for the networks had the same lengths as given above.

Uncorrected *p* distances and isolation by distance for mtDNA: Uncorrected *p* distances were calculated for the mitochondrial cyt *b* gene alone as well as for the mtDNA alignment of concatenated sequences using MEGA 7.0.21 (Kumar et al. 2016) and the pairwise deletion option. The distances of the concatenated sequence data were used to examine for a positive correlation between geographic and genetic distances (isolation by distance). For this purpose, Mantel tests as implemented in IBD 1.52 (Bohonak 2002) were run for three data sets using genetic and spatial distances. The latter were obtained via the Geographic Distance Matrix Generator 1.2.3 (http://biodiversityinformatics.amnh.org/open_source/gdmg/index.php). The significance of the slope of the reduced major axis (RMA) regression was assessed by 30,000 randomizations. One data set included the sequences for all 61 terrapins. The other two data sets included the sequences for each clade of *P. sinuatus* (clades A and B) identified in the present study.

Table 1. Partitioning and evolutionary models used for MrBayes.

Subset	nst	rates	Model
1-398 401-1311\3 1314-1998\3 1999-2180	6	gamma	SYM + gamma
399-1311\3 1312-1998\3	6		SYM
400-1311\3 1313-1998\3	6	gamma	SYM + gamma

Body size: Using Vernier calipers (accuracy 0.1 mm), straight carapace length of adult terrapins was recorded as a measure of body size during fieldwork in South Africa ($n = 11$). Also measured were specimens in the Museum for Comparative Zoology, Cambridge, Massachusetts, USA (MCZ 39383, South Africa), the Museum für Naturkunde, Berlin, Germany (ZMB 158, Mozambique; ZMB 5517, 5518, 15689, 16158, 16242, Tanzania; ZMB 22416, Burundi), the Museum für Tierkunde (Museum of Zoology), Senckenberg, Dresden, Germany (MTD D 49650, South Africa), and the Paläontologisches Institut und Museum, Zürich, Switzerland (PIMUZ A/III527, Tanzania). Measurements were divided into northern and southern groups according to the two genetic clades identified in the present study. Since males and females could not be distinguished in all samples, the two sexes were combined for analysis. After testing for whether the data were parametric, the body sizes of the groups were compared using a *t*-test as implemented in SigmaPlot 13.0 (Systat Software, Inc., San Jose, California, USA).

Results

Phylogenetic and haplotype network analyses of mtDNA: Both tree-building approaches delivered the same topology, corresponding to two geographically widespread clades, A and B, both of which showed substructuring and received high support values (Fig. 1). Clade A corresponded to the samples from the north and center of the distribution range of *Pelusios sinuatus* (Tanzania, Mozambique, and Botswana), and clade B to samples from the south (Botswana and South Africa). Clade A consisted of three subclades and clade B, of two. All subclades of clade A and one subclade of clade B were well supported under both Bayesian and ML analyses; the second subclade of clade B was moderately supported. In Botswana and northeastern South Africa, records of terrapins representing clades A and B are only separated by a distance of approximately 200 km, and in northeastern South Africa, representatives of subclades B1 and B2 were found in two sites syntopically.

In haplotype networks of the three mtDNA fragments, no shared haplotypes occurred for the two clades (Fig. 2). Four 12S haplotypes were found for clade A, with two private haplotypes for subclade A2 that differed by one mutation step each from a shared haplotype that included sequences of subclades A1 and A3. Another private haplotype of subclade A3 also differed by one mutation step from the previously mentioned shared haplotype. This shared haplotype was separated by three mutation steps from a common haplotype containing sequences of subclades B1 and B2, and a second rare haplotype for subclade B1 differed by one mutation step from this common haplotype. Haplotype networks of the two other mtDNA fragments showed more differentiation, with no shared haplotypes between any clades or subclades. For the *cyt b* fragment, haplotypes of clades A and B were separated by a minimum of 21 steps. Within haplotypes of clade A, up to 10 mutations occurred, with each subclade corresponding to one distinct haplotype. Within haplotypes of clade B, a loop occurred that connected three of the four haplotypes of subclade B1;

and the four haplotypes of this subclade were separated by a minimum of four steps from the three haplotypes of subclade B2, each of which differed by one mutation step. With respect to the mtDNA fragment containing the partial ND4 gene and adjacent DNA coding for tRNAs, haplotypes of clades A and B differed by a minimum of 13 mutations. Subclade A1 was represented by one haplotype. Subclade A2 consisted of three haplotypes that each differed by a maximum of three mutations; and subclade A3 had four haplotypes that differed by up to four steps. Within the individual haplotypes of clade A, a maximum of nine steps occurred, and the three subclades were separated by a minimum of 4–7 steps. The three haplotypes of subclade B1 differed by a maximum of two mutations and the two haplotypes of subclade B2, by one mutation, and the two subclades were distinct by a minimum of six steps.

Haplotype network analyses of nuclear loci: The three nuclear loci showed distinctly less variation compared to the mtDNA fragments. Often, shared haplotypes between distinct clades and subclades were found (Fig. 3). For the *Cmos* gene, the 15 haplotypes found differed by a maximum of nine mutation steps. Clades A and B shared two haplotypes, even though the vast majority of phased sequences of clades A and B corresponded to unique haplotypes for each clade. A generally similar picture was revealed for the *Rag2* locus, with one shared haplotype of clade A and clade B, six unique additional haplotypes of clade A, and three further unique haplotypes of clade B. The maximum number of mutations between the *Rag2* haplotypes was seven. For intron 1 of the *R35* gene, no shared haplotypes were found for clades A and B. A total of 15 haplotypes occurred that were partially connected over a loop. In a direct line (not across the loop), the haplotypes differed by up to seven mutations. Of the 15 haplotypes, six corresponded to clade A and nine to clade B.

Uncorrected *p* distances and isolation by distance for mtDNA: Sequence divergences of the mitochondrial *cyt b* gene are often used to distinguish between chelonian taxa (e.g., Iverson et al. 2013; Kindler et al. 2012, 2016; Petzold et al. 2014). The uncorrected *p* distance between clade A and clade B of *P. sinuatus* amounted to 2.80% on average, while the within-group values were 1.05% and 0.28%, respectively. Between the individual subclades of clade A, divergences ranged between 1.31% and 1.54%, with no variation within those two clades for which sequences of more than one individual were available. Subclades B1 and B2 differed by only 0.57%, with within-group divergences of 0.06% and 0.02%, respectively (Table 2).

The IBD test for all data revealed a statistically significant correlation of genetic and geographic distances ($Z = 22487528$, $r = 0.65$, $p \leq 0.0001$; $n = 61$). When the two clades were analyzed separately, a statistically significant correlation was also found for clade A ($Z = 1387859$, $r = 0.58$, $p \leq 0.0003$; $n = 17$), but not for clade B ($Z = 9009469796357$, $r = 0.06$, $p \leq 0.0777$; $n = 44$).

Body size: The mean straight carapace length ($\pm$ SD) for the samples of the northern and southern clades,

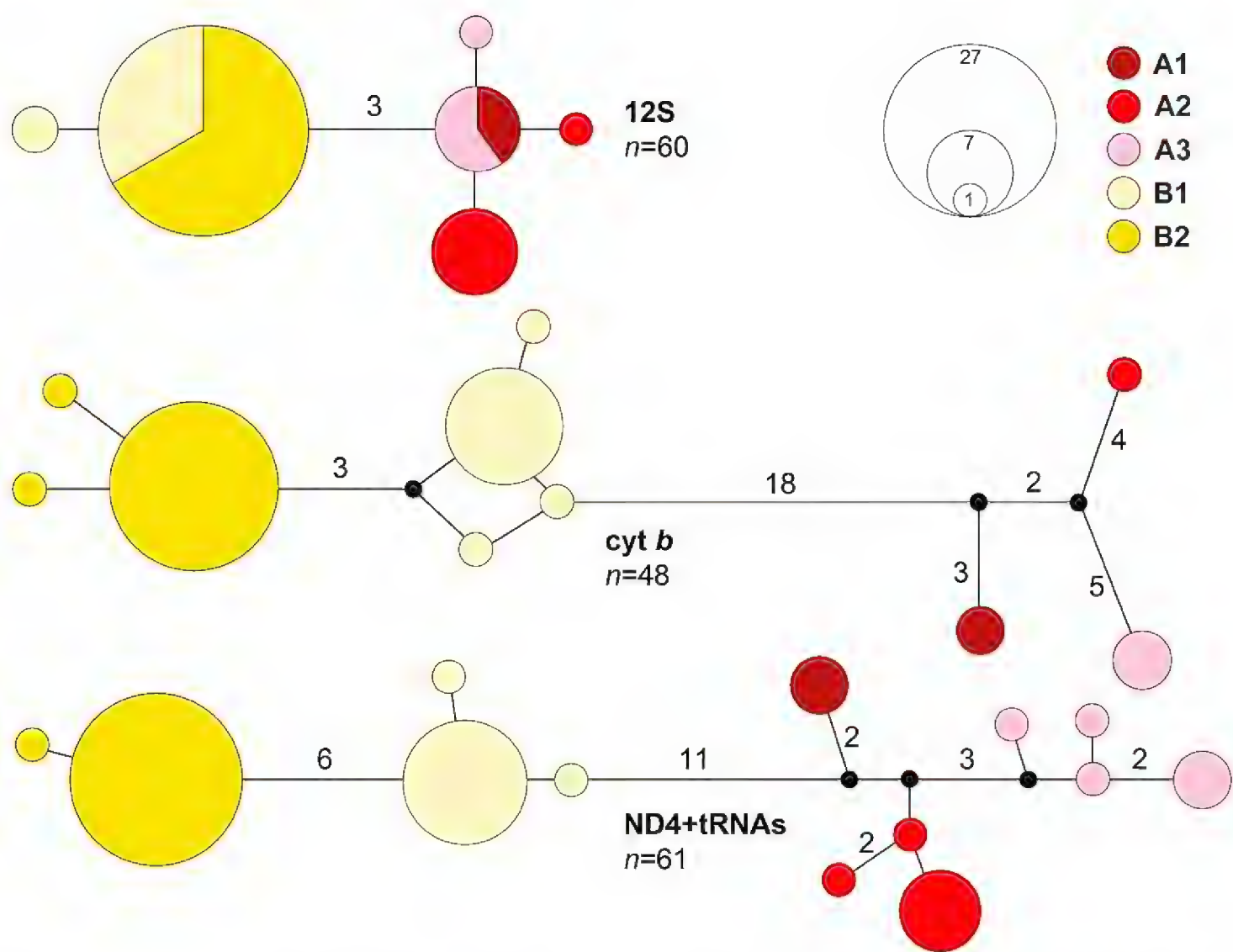


Fig. 2. Parsimony networks for individual mtDNA fragments of *Pelusios sinuatus*. Symbol size is proportional to haplotype frequency. Each line connecting two haplotypes corresponds to one mutation step, if not otherwise indicated by numbers of substitutions along the lines. Colors correspond to Fig. 1. Small black circles represent missing node haplotypes.

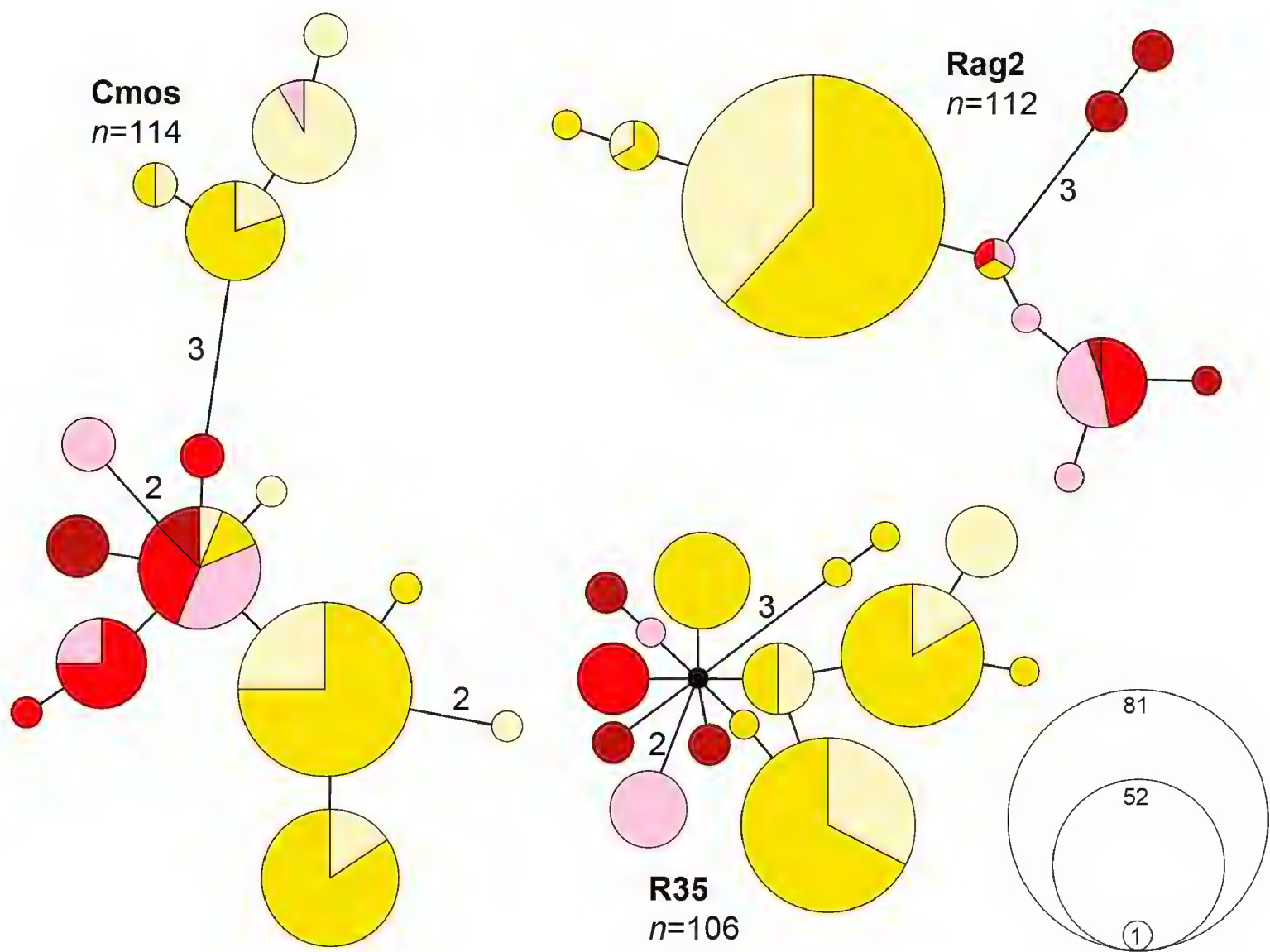


Fig. 3. Parsimony networks for nuclear loci of *Pelusios sinuatus*. Symbol size is proportional to haplotype frequency. Each line connecting two haplotypes corresponds to one mutation step, if not otherwise indicated by numbers of substitutions along the lines. Colors correspond to Fig. 1. The small black circle represents a missing node haplotype. Sample sizes refer to phased nuclear sequences, i.e., each individual is represented twice.

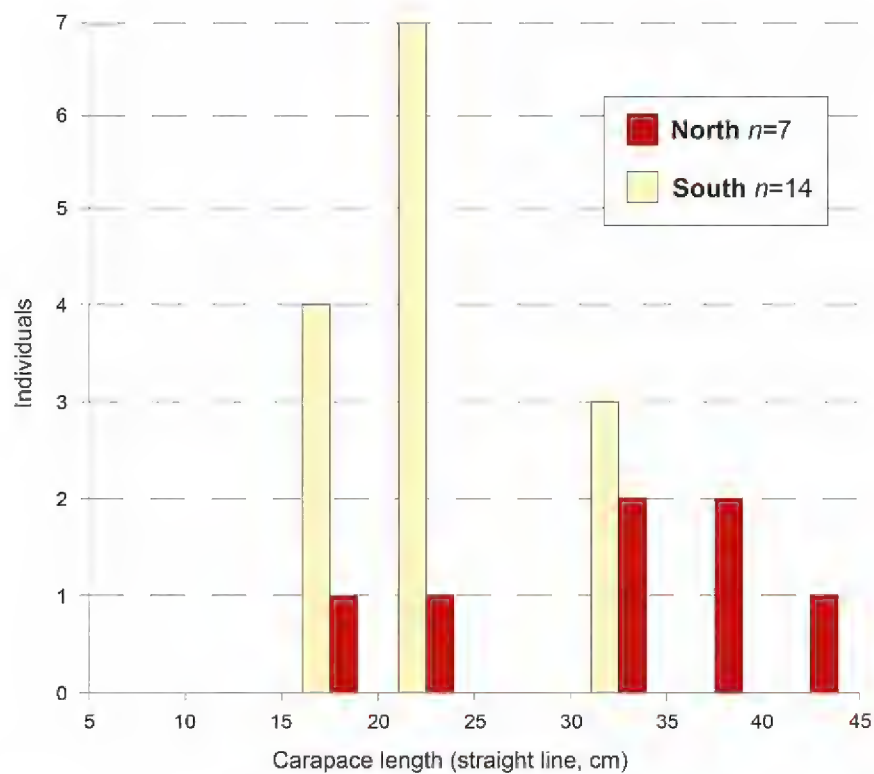


Fig. 4. Carapace lengths of adult *Pelusios sinuatus* from the northern and the southern distribution range (museum specimens and wild-caught terrapins), corresponding to clades A (north) and B (south).

respectively, were 31.8 ± 7.9 cm ($n = 7$) and 24.0 ± 5.5 cm ($n = 14$), with terrapins from the northern clade being significantly larger than those from the southern clade ($t_{19} = 2.66$, $p = 0.0156$).

Discussion

The present study is the first assessment of the phylogeography for the Serrated Hinged Terrapin (*Pelusios sinuatus*), which is widely distributed in East Africa (TTWG 2017; inset in Fig. 1). In north-south direction, the distribution area extends over approximately 3,500 km and in east-west direction, over more than 1,500 km. Within this large area, two mitochondrial clades (A and B) with parapatric distribution and substantial geographic substructure were discovered (Figs. 1 and 2). In contrast to mitochondrial DNA, the slower evolving nuclear DNA has not reached complete lineage sorting for the Cmos and Rag2 loci (Fig. 3), even though haplotype sharing between clades A and B was restricted. For intron 1 of the R35 gene, no shared haplotypes occurred. Thus, mitochondrial and nuclear markers show largely concordant differentiation patterns.

Clade A was found in the northern and central parts of the distribution range (Tanzania, Mozambique, and Botswana), and clade B, in the south (Botswana and South Africa). Close to the border region of Botswana, Zimbabwe, and South Africa the two clades abut, which explains why Fritz et al. (2011) had already discovered the two clades using only three samples from that region. Nearby, to the southeast, sites were found with syntopic occurrences of the two otherwise parapatric mitochondrial subclades within clade B. This implies that the correlation of genetic and geographic distances for the whole data set cannot result from isolation by distance alone, because then neither distinct clades nor contact zones would be expected (Figs. 1 and 2). This is also supported by the absence of evidence for isolation by distance in the southern clade B. Therefore, we conclude

Table 2. Uncorrected p distances (means, expressed as percentages) between and within mitochondrial subclades of *Pelusios sinuatus* using the cyt b gene (913 bp). Below the diagonal are between-group values; on the diagonal, within-group divergences are in bold.

	<i>n</i>	A1	A2	A3	B1	B2
A1	2	0				
A2	1	1.31	—			
A3	3	1.53	1.31	0		
B1	15	2.83	2.85	2.63	0.06	
B2	27	2.75	2.97	2.75	0.57	0.02

that the observed genetic divergence is, at least in part, caused by vicariance and subsequent dispersal, and that the correlation of geographic and genetic distances results mainly from our patchy sampling. A future challenge is to close the large sampling gaps in order to locate additional contact zones, especially between the northern subclades.

The north-south differentiation of *P. sinuatus* is similar to that in another terrapin species. *Pelusios castanoides* has a continental distribution range similar to *P. sinuatus* but occurs also on Madagascar and the Seychelles (TTWG 2017), although it is unclear whether the latter populations are native. In *P. castanoides*, a sample from the north of the distribution range (Kenya) was distinct from samples from South Africa and southern Mozambique (Fritz et al. 2013), suggesting a shared biogeographic history with *P. sinuatus*.

To the best of our knowledge, *P. sinuatus* from different parts of the distribution range have never been compared morphologically, but northern terrapins (clade A) seem to grow to a larger maximum size than southern ones (clade B). According to de Witte (1952), *P. sinuatus* reaches 46.5 cm in Lake Tanganyika. Branch (2008) reports a maximum shell length of 48.5 cm for *P. sinuatus*, and Spawls et al. (2002) mention up to 55 cm for upland Kenya. Serrated Hinged Terrapins of such size are never seen in South Africa. This is confirmed by the measurements of wild animals and collection material reported here (Fig. 4) that show a statistically significant difference between the mean carapace lengths of terrapins representing clades A and B. Seven adult museum specimens from Tanzania and Burundi (clade A) had straight carapace lengths from 19.6 cm to 40.0 cm (i.e., the largest specimens are still distinctly below the published maximum size). In contrast, 14 adult museum specimens and wild terrapins from the distribution range of clade B (Mozambique and South Africa) ranged between 17.5 cm and 34.7 cm. Thus, it appears that northern and southern terrapins differ morphologically, at least with respect to their maximum size, but more measurements are required and further studies warranted for comparing variations in additional morphological characters. The size variation of *P. sinuatus* is reminiscent of other turtles in which size increases with latitude (Ashton and Feldman 2003), either within the same species (e.g., *Chelonoidis chilensis*: Fritz et al. 2012a; *Testudo graeca*: Werner et al. 2016) or in distinct species of the same genus (*Pelodiscus* spp.: Farkas et al. 2019). However, there are exceptions. For instance, in Leopard Tortoises (*Stigmochelys*

Table 3. Average uncorrected *p* distances (percentages) of 795 bp of the *cyt b* gene of *Pelusios* species from Kindler et al. (2016). *Pelusios subniger* includes a putative undescribed species from the Democratic Republic of the Congo (n = 2). It differs from other *P. subniger* by an average distance of 3.13% (Petzold et al. 2014). The relationship of *P. carinatus* and *P. rhodesianus* is unclear; some populations assigned to the latter species (*P. rhodesianus* A) could be conspecific with *P. carinatus* (Kindler et al. 2016). Values for sympatrically occurring species pairs in boldface and red.

	<i>n</i>	<i>ada</i>	<i>bec</i>	<i>bro</i>	<i>car</i>	<i>c'us</i>	<i>c'es</i>	<i>cha</i>	<i>cup</i>	<i>gab</i>	<i>mar</i>	<i>nan</i>	<i>nig</i>	<i>rho A</i>	<i>rho B</i>	<i>sin</i>	<i>sub</i>	<i>upe</i>	<i>wil</i>
<i>adansonii</i>	1																		
<i>bechuanicus</i>	2	11.59																	
<i>broadleyi</i>	7	3.05	13.15																
<i>carinatus</i>	15	9.92	12.91	11.17															
<i>castaneus</i>	19	6.52	13.70	7.55	11.48														
<i>castanoides</i>	29	9.57	11.50	9.88	10.99	11.17													
<i>chapini</i>	8	6.03	15.91	10.17	13.95	4.34	12.35												
<i>cupulatta</i>	4	10.98	10.79	11.24	11.26	12.14	10.76	15.03											
<i>gabonensis</i>	24	12.73	13.62	13.68	12.84	13.52	12.54	12.42	13.09										
<i>marani</i>	5	12.80	11.21	12.68	13.01	13.57	12.50	15.35	11.82	13.75									
<i>namus</i>	26	11.59	14.99	15.07	15.31	15.36	12.21	12.94	14.09	12.42	15.25								
<i>niger</i>	2	11.76	13.38	13.90	12.25	15.17	11.85	15.77	9.10	12.73	14.14	13.07							
<i>rhodesianus</i> A	6	10.81	13.45	11.39	2.49	11.83	11.12	10.92	12.28	11.43	14.19	13.24	12.57						
<i>rhodesianus</i> B	14	7.93	14.23	11.93	6.22	12.39	12.12	10.63	13.44	12.09	14.58	12.41	12.77	4.04					
<i>sinuatus</i>	2	14.02	11.42	13.05	12.52	14.01	13.60	16.32	11.26	13.95	11.02	15.73	14.34	13.92	13.96				
<i>subniger</i>	41	14.57	5.33	12.25	12.70	13.50	11.89	15.28	10.92	13.15	11.21	13.72	11.64	13.50	13.55	10.08			
<i>upembae</i>	3	10.98	1.38	13.15	13.04	14.41	11.50	16.54	10.91	14.01	11.82	14.99	13.56	14.56	13.54	11.80	5.43		
<i>williamsi</i>	2	9.15	10.91	10.87	10.23	11.03	3.89	15.00	11.01	14.11	11.33	15.73	13.69	13.97	10.77	13.31	12.28	11.31	

pardalis) the northernmost and southernmost populations comprise large-sized individuals, while tortoises from geographically intermediate populations are medium-sized (Fritz et al. 2010; Spitzweg et al. 2019). Another pattern is found in continental *Trachemys* species once considered conspecific, with taxa having the largest body sizes in Central America and distinctly smaller-sized North and South American congeners (Ernst and Barbour 1989; Legler and Vogt 2013; Vargas-Ramírez et al. 2017). Clearly, further research is needed for a better understanding of the described size variation in *P. sinuatus* and other turtle species, but we concur with Joos et al. (2017) and Spitzweg et al. (2019) that many factors beyond latitude act in concert on such variation.

An open question remains how the genetic and morphological differentiation patterns of *P. sinuatus* relate to taxonomy. The concordant variation of different genetic and morphological characters justifies recognizing each clade within *P. sinuatus* as a distinct taxon. However, without entering the debate about species concepts and species conceptualization (e.g., de Queiroz 2007; Zachos 2016), we are reluctant to assign species status to either clade. In our understanding, restricted gene flow and largely isolated gene pools represent unambiguous traits of distinct species. In contrast to other cases (e.g., Kindler et al. 2017; Spinks et al. 2014; Vamberger et al. 2015), patchy sampling prevents sound conclusions here, particularly the lack of comprehensive sampling from the putative contact zone of clades A and B. Yet, in times when legislative restrictions make biodiversity research virtually impossible for many widely distributed species (Neumann et al. 2018; Prathapan et al. 2018), researchers are often forced to use the evidence available as a starting point.

The mitochondrial divergence of the two clades (Figs. 1 and 2), together with concordant variation in the nuclear loci (Fig. 3), provide two important insights: (1) the two mitochondrial clades represent distinct genealogical lineages; and (2) mitochondrial introgression plays no obvious role here, allowing the application of mtDNA to infer taxonomic differentiation. Uncorrected *p* distances of the mitochondrial *cyt b* gene have frequently been used as a ‘taxonomic yardstick’ to decide which taxonomic rank should be applied to turtle taxa (e.g., Iverson et al. 2013; Kindler et al. 2012, 2016; Petzold et al. 2014; Spinks et al. 2004), analogous to the widely applied barcoding approach (e.g., Hebert et al. 2003). However, as pointed out by Fritz et al. (2012b) and Kindler et al. (2012), the wide range of genetic divergences between different turtle species (differing by one order of magnitude) prevents the application of a rigid threshold across all turtle groups. Instead, thresholds for different groups need to be adjusted individually using unambiguous, ideally sympatric, species that are closely related to the taxa in question. Thus, previously published *cyt b* data for other *Pelusios* species (Kindler et al. 2016; Petzold et al. 2014) can serve here for comparison. Also, for these species a wide range has been reported (pairwise average divergences between species vary from 1.38% to 16.54%), even though the low value of 1.38% between the allopatric *P. bechuanicus* and *P. upembae* has been suggested to indicate their conspecificity (Kindler et al. 2016). When only divergences of sympatric species are considered, the values range between 2.49% and 15.35% (Table 3). Yet, the lowest value refers to *P. carinatus* and populations of *P. rhodesianus* that could actually be conspecific with *P. carinatus* (Kindler et al. 2016).

If this value is disregarded, the lowest value between unambiguous sympatric species amounts to 5.43% (*P. subniger* vs. *P. upembae*), and this value is much higher than the divergence between clades A and B of *P. sinuatus* (2.80%) found here.

In view of this relatively low value, we suggest subspecies status for the Serrated Hinged Terrapins from the southernmost and more northerly parts of the distribution range. The name *Sternotherus sinuatus* Smith, 1838 is clearly referable to the southern subspecies, while the oldest name for the northern subspecies is *Sternotherus bottegi* Boulenger, 1895 (Fig. 1). Accordingly, the smaller-sized southern populations represent the nominotypical subspecies *Pelusios sinuatus sinuatus* (Smith, 1838), and the large-sized northern subspecies is to be named *Pelusios sinuatus bottegi* (Boulenger, 1895) nov. comb. Another name, *Pelusios sinuatus zuluensis* Hewitt, 1927 (type locality: Mzinene River, KwaZulu-Natal, South Africa) clearly is a junior synonym of the nominotypical subspecies. A fourth name, *Pelusios sinuatus leptus* Hewitt, 1933 (type locality: Isoka, Zambia) can be identified with the northern subspecies, and is thus a junior synonym of *P. s. bottegi*.

This assessment is in line with the recent proposal to use the subspecies category for naming lineages that qualify for the genetic criteria of Evolutionarily Significant Units (ESUs; Moritz 1994). Accordingly, subspecies should correspond to distinct mtDNA lineages (except for cases of mitochondrial capture), and they should be diagnosable by nuclear genomic evidence. However, in contrast to species, subspecies are genetically less divergent and capable of large-scale gene flow with other subspecies. Applying subspecies names for such lineages facilitates communication within and beyond science, particularly in legislation and conservation (Kindler and Fritz 2018). In this vein, the recognition of two subspecies of *P. sinuatus* not only reflects their genetic divergence but also will contribute in the medium term to their conservation. Currently, *P. sinuatus* is not considered to be imperiled (IUCN category “Least Concern,” Rhodin et al. 2018). However, in many African countries freshwater habitats are increasingly threatened by progressing land use and, consequently, the numbers of Serrated Hinged Terrapins are dwindling. Furthermore, we propose to treat the subclades A1–A3 within *P. s. bottegi* and subclades B1 and B2 within *P. s. sinuatus* as distinct Management Units in the sense of Moritz (1994), i.e., as populations with significant mitochondrial divergence.

Conclusions

Serrated Hinged Terrapins (*Pelusios sinuatus*) show concordant variation in mitochondrial and nuclear marker genes, corresponding to two distinct genealogical lineages in the southernmost and more northerly parts of the distribution range. Each lineage displays phylogeographic structuring. Terrapins representing the two lineages differ also in body size, with individuals from the northern and central parts of the distribution reaching larger sizes than terrapins from the southern parts. Considering the degree of genetic differentiation

compared to other *Pelusios* species, we conclude that the two lineages should be regarded as distinct subspecies. The nominotypical subspecies *Pelusios sinuatus sinuatus* (Smith, 1838) corresponds to populations in the south (South Africa and parts of Botswana), and the resurrected taxon *Pelusios sinuatus bottegi* (Boulenger, 1895) nov. comb. to populations from the northern and central distribution range. A contact zone of the two subspecies is identified in the border region of Botswana, South Africa, and Zimbabwe. The genetically differentiated population clusters within each subspecies should be treated as distinct Management Units. Further research is needed to find out whether additional diagnostic morphological characters for the two subspecies exist. In addition, denser sampling would allow a fine-scale phylogeography for the species including an assessment of gene flow between the two subspecies and the Management Units within each subspecies. Such research could contribute significantly to the development of long-term management plans for this species. However, the current legislative situation makes progress unlikely because multiple countries are involved and obtaining sampling permits for biodiversity research often becomes a major, if not insurmountable, administrative obstacle.

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Literature Cited

- Ashton KG, Feldman CR. 2003. Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes inverse it. *Evolution* 57: 1,151–1,163.
- Bohonak AJ. 2002. IBD (isolation by distance): a program for analyses of isolation by distance. *Journal of Heredity* 93: 153–154.
- Bramble DM, Hutchison JH. 1981. A reevaluation of plastral kinesis in African turtles of the genus *Pelusios*. *Herpetologica* 37: 205–212.
- Branch B. 2008. *Tortoises, Terrapins & Turtles*. Struik Publishers, Cape Town, South Africa. 128 p.
- Broadley DG. 1981. A review of the genus *Pelusios* Wagler in southern Africa (Pleurodira: Pelomedusidae). *Occasional Papers of the National Museums and Monuments of Rhodesia, Series B, Natural Sciences* 6: 633–686.
- Broadley DG, Boycott RC. 2009. *Pelusios sinuatus* (Smith 1838) – serrated hinged terrapin. Pp. 036.1–036.5 In: *Conservation Biology of Freshwater Turtles and Tortoises: a Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group*. Chelonian Research Monographs 5. Editors, Rhodin AGJ, Pritchard PCH, van Dijk PP, Saumure RA, Buhlmann KA, Iverson JB, Mittermeier RA. Chelonian Research Foundation, Lunenburg, Massachusetts, USA. Loose-leaf (pagination variable).
- Calabresi E. 1916. Batraci e rettili raccolti nella Somalia meridionale dai Dott. Stefani e Paoli. *Monitore Zoologico Italiano* 27: 33–45, plate ii.
- Curat M, Ruedi M, Petit RJ, Excoffier L. 2009. The hidden side of invasions: massive introgression by local genes. *Evolution* 62: 1,908–1,920.
- de Broin F. 1988. Les tortues et le Gondwana. Examen des rapports entre le fractionnement du Gondwana et la dispersion géographique des tortues pleurodires à partir du Crétacé. *Studia Geologica Salmanticensis* 2 (*Studia Palaeocheloniologica* II): 103–142.
- de Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56: 879–886.
- de Witte GF. 1952. *Amphibiens et Reptiles. Exploration Hydrobiologique du Lac Tanganyika (1946-1947)*. Volume III, fascicule 3. Ministère des Colonies, Institut Royal des Sciences Naturelles de Belgique, Bruxelles, Belgium. 22 p., 1 plate.
- Ernst CH, Barbour RW. 1989. *Turtles of the World*. Smithsonian Institution Press, Washington, DC, USA, and London, United Kingdom. 313 p.
- Farkas B, Ziegler T, Pham CT, Ong AV, Fritz U. 2019. A new species of *Pelodiscus* (Testudines, Trionychidae) from northeastern Indochina. *ZooKeys* 824: 71–86.
- Fritz U, Alcalde L, Vargas-Ramírez M, Goode EV, Fabius-Turoblin DU, Praschag P. 2012a. Northern genetic richness and southern purity, but just one species in the *Chelonoidis chilensis* complex. *Zoologica Scripta* 41: 220–232.
- Fritz U, Branch WR, Gehring PS, Harvey J, Kindler C, Meyer L, Du Preez L, Široký P, Vieites DR, Vences M. 2013. Weak divergence among African, Malagasy and Seychellois hinged terrapins (*Pelusios castanoides*, *P. subniger*) and evidence for human-mediated overseas dispersal. *Organisms Diversity & Evolution* 13: 215–224.
- Fritz U, Branch WR, Hofmeyr MD, Maran J, Prokop H, Schleicher A, Široký P, Stuckas H, Vargas-Ramírez M, Vences M, et al. 2011. Molecular phylogeny of African hinged and helmeted terrapins (Testudines: Pelomedusidae: *Pelusios* and *Pelomedusa*). *Zoologica Scripta* 40: 115–125.
- Fritz U, Daniels SR, Hofmeyr MD, González J, Barrio-Amorós CL, Široký P, Hundsdoerfer AK, Stuckas H. 2010. Mitochondrial phylogeography and subspecies of the wide-ranging sub-Saharan leopard tortoise *Stigmochelys pardalis* (Testudines: Testudinidae) – a case study for the pitfalls of pseudogenes and GenBank sequences. *Journal of Zoological Systematics and Evolutionary Research* 48: 348–359.
- Fritz U, Stuckas H, Vargas-Ramírez M, Hundsdoerfer AK, Maran J, Päckert M. 2012b. Molecular phylogeny of Central and South American slider turtles: implications for biogeography and systematics (Testudines: Emydidae: *Trachemys*). *Journal of Zoological Systematics and Evolutionary Research* 50: 125–136.
- Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics* 34: 397–423.
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hebert PDN, Ratnasingham S, de Waard JR. 2003. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society London B* 270 (Supplement): S96–S99.
- Hewitt J. 1927. Further descriptions of reptiles and batrachians from South Africa. *Records of the Albany Museum* 3: 371–415, plates XX–XXIV.
- Hewitt J. 1933. Descriptions of some new reptiles and a frog from Rhodesia. *Occasional Papers of the Rhodesian Museums* 1: 45–50.
- Iverson JB, Le M, Ingram C. 2013. Molecular phylogenetics of the mud and musk turtle family Kinosternidae. *Molecular Phylogenetics and Evolution* 69: 929–939.
- Joos J, Kirchner M, Vamberger M, Kaviani M, Rahimibashar MR, Fritz U, Müller J. 2017. Climate and patterns of body size variation in the European

- pond turtle, *Emys orbicularis*. *Biological Journal of the Linnean Society* 122: 351–365.
- Kehlmaier C, Zhang X, Georges A, Campbell PD, Thomson S, Fritz U. 2019. Mitogenomics of historical type specimens of Australasian turtles: clarification of taxonomic confusion and old mitochondrial introgression. *Scientific Reports* 9: 5,841.
- Kindler C, Branch WR, Hofmeyr MD, Maran J, Široký P, Vences M, Harvey J, Hauswaldt JS, Schleicher A, Stuckas H, et al. 2012. Molecular phylogeny of African hinge-back tortoises (*Kinixys*): implications for phylogeography and taxonomy (Testudines: Testudinidae). *Journal of Zoological Systematics and Evolutionary Research* 50: 192–201.
- Kindler C, Chèvre M, Ursenbacher S, Böhme W, Hille A, Jablonski D, Vamberger M, Fritz U. 2017. Hybridization patterns in two contact zones of grass snakes reveal a new Central European snake species. *Scientific Reports* 7: 7,378.
- Kindler C, Fritz U. 2018. Phylogeography and taxonomy of the barred grass snake (*Natrix helvetica*), with a discussion of the subspecies category in zoology. *Vertebrate Zoology* 68: 269–281.
- Kindler C, Moosig M, Branch WR, Harvey J, Kehlmaier C, Nagy ZT, Prokop H, Široký P, Fritz U. 2016. Comparative phylogeographies of six species of hinged terrapins (*Pelusios* spp.) reveal discordant patterns and unexpected differentiation in the *P. castaneus*/*P. chapini* complex and *P. rhodesianus*. *Biological Journal of the Linnean Society* 117: 305–321.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1,870–1,874.
- Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1,695–1,701.
- Legler JM, Vogt RC. 2013. *The Turtles of Mexico. Land and Freshwater Forms*. University of California Press, Berkeley and Los Angeles, California, USA, and London, United Kingdom. 402 p.
- Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1,451–1,452.
- Loveridge A. 1936. Scientific results of an expedition to rain forest regions in Eastern Africa. V. Reptiles. *Bulletin of the Museum of Comparative Zoology* 79: 209–337, plates i–ix.
- Moritz C. 1994. Defining ‘Evolutionarily Significant Units’ for conservation. *Trends in Ecology & Evolution* 9: 373–375.
- Neumann D, Borisenko AV, Coddington JA, Häuser CL, Butler CR, Casino A, Vogel JC, Haszprunar G, Giere P. 2018. Global biodiversity research tied up by juridical interpretations of access and benefit sharing. *Organisms Diversity & Evolution* 18: 1–18.
- Noonan BP. 2000. Does the phylogeny of pelomedusoid turtles reflect vicariance due to continental drift? *Journal of Biogeography* 27: 1,246–1,249.
- Petzold A, Vargas-Ramírez M, Kehlmaier C, Vamberger M, Branch WR, Du Preez L, Hofmeyr MD, Meyer L, Schleicher A, Široký P, et al. 2014. A revision of African helmeted terrapins (Testudines: Pelomedusidae: *Pelomedusa*), with descriptions of six new species. *Zootaxa* 3795: 523–548.
- Prathapan KD, Pethiyagoda R, Bawa KS, Raven PH, Rajan D. 2018. When the cure kills – CBD limits biodiversity research. National laws fearing biopiracy squelch taxonomy studies. *Science* 360: 1,405–1,406.
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67: 901–904.
- Rhodin AGJ, Stanford CB, van Dijk PP, Eisemberg C, Luiselli L, Mittermeier RA, Hudson R, Horne BD, Goode EV, Kuchling G, et al. 2018. Global conservation status of turtles and tortoises (order Testudines). *Chelonian Conservation and Biology* 17: 135–161.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Siebenrock F. 1916. Schildkröten aus dem nördlichen Seengebiet und von Belgisch-Kongo. *Annalen des Naturhistorischen Museums Wien* 30: 1–12, plates 1–2.
- Sloan DB, Havird JC, Sharbrough J. 2017. The on-again, off-again relationship between mitochondrial genomes and species boundaries. *Molecular Ecology* 26: 2,212–2,236.
- Spawls S, Howell K, Drewes R, Ashe J. 2002. *A Field Guide to the Reptiles of East Africa: Kenya, Tanzania, Uganda, Rwanda and Burundi*. Academic Press, San Diego, California, USA. 543 p.
- Spinks PQ, Shaffer HB, Iverson JB, McCord WP. 2004. Phylogenetic hypotheses for the turtle family Geoemydidae. *Molecular Phylogenetics and Evolution* 32: 164–182.
- Spinks PQ, Thomson RC, Shaffer HB. 2014. The advantages of going large: genome-wide SNPs clarify the complex population history and systematics of the threatened western pond turtle. *Molecular Ecology* 23: 2,228–2,241.
- Spitzweg C, Hofmeyr MD, Fritz U, Vamberger M. 2019. Leopard tortoises in southern Africa have greater genetic diversity in the north than in the south (Testudinidae). *Zoologica Scripta* 48: 57–68.
- Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2,688–2,690.
- Stuckas H, Gemel R, Fritz U. 2013. One extinct turtle species less: *Pelusios seychellensis* is not extinct, it never existed. *PLoS ONE* 8: e57116.
- Toews DPL, Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology* 21: 3,907–3,930.
- TTWG [Turtle Taxonomy Working Group, Rhodin AGJ,

Iverson JB, Fritz U, Georges A, Shaffer HB, van Dijk PP]. 2017. *Turtles of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status*. 8th Edition. Chelonian Research Monographs 7. Chelonian Research Foundation and Turtle Conservancy, Lunenburg, Massachusetts, USA. 292 p.

Vamberger M, Stuckas H, Sacco F, D’Angelo S, Arculeo M, Cheylan M, Corti C, Lo Valvo M, Marrone F, Wink M, et al. 2015. Differences in gene flow in a twofold secondary contact zone of pond turtles in southern Italy (Testudines: Emydidae: *Emys orbicularis galloitalica*, *E. o. hellenica*, *E. trinacris*). *Zoologica Scripta* 44: 233–249.

Vargas-Ramírez M, del Valle C, Ceballos CP, Fritz U. 2017. *Trachemys medemi* n. sp. from northwestern Colombia turns the biogeography of South American slider turtles upside down. *Journal of Zoological Systematics and Evolutionary Research* 55: 326–339.

Wermuth H, Mertens R. 1961. *Schildkröten, Krokodile, Brückenechsen*. VEB Gustav Fischer, Jena, Germany. 422 p.

Wermuth H, Mertens R. 1977. Testudines, Crocodylia, Rhynchocephalia. *Das Tierreich* 100: i–xxvii, 1–174.

Werner YL, Korolker N, Sion G, Göçmen B. 2016. Bergmann’s and Rensch’s rules and the spur-thighed tortoise (*Testudo graeca*). *Biological Journal of the Linnean Society* 117: 796–811.

Zachos FE. 2016. *Species Concepts in Biology. Historical Development, Theoretical Foundations and Practical Relevance*. Springer International Publishing Switzerland, Basel, Switzerland. 220 p.

Appendix 1. Studied material of *Pelusios sinuatus* and outgroups and European Nucleotide Archive (ENA) accession numbers. Accession numbers starting with LR correspond to sequences produced for the present study.

Sample	12S	cyt <i>b</i>	ND4	Cmos	R35	Rag2	Provenance	Latitude	Longitude	mtDNA subclade
14050	LR594053	LR594111	LR594157	LR594218	LR594272	LR594325	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14051	LR594054	LR594112	LR594158	LR594219	LR594273	LR594326	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14052	LR594055	LR594113	LR594159	LR594220	LR594274	LR594327	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14053	LR594056	LR594114	LR594160	LR594221	LR594275	LR594328	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14054	LR594057	LR594115	LR594161	LR594222	LR594276	LR594329	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14055	LR594058	LR594116	LR594162	LR594223	LR594277	LR594330	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
14056	LR594059	LR594117	LR594163	LR594224	LR594278	LR594331	Botswana: Goo-Moremi Gorge	-22.58402	27.43988	B1
5564	FR716875	n/a	FR716984	FR717028	FR717076	FR717121	Botswana: Mashatu Game Reserve	-22.212308	29.136038	A1
5565	LR594060	n/a	LR594164	LR594225	LR594279	LR594332	Botswana: Mashatu Game Reserve	-22.212308	29.136038	A1
7044	LR594061	n/a	LR594165	LR594226	LR594280	LR594333	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
7045	LR594062	n/a	LR594166	LR594227	n/a	n/a	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
7046	LR594063	n/a	LR594167	LR594228	n/a	LR594334	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
7047	LR594064	n/a	LR594168	LR594229	n/a	n/a	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
7048	LR594065	n/a	LR594169	n/a	n/a	n/a	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
7049	LR594066	n/a	LR594170	LR594230	n/a	LR594335	Mozambique: Cabo Delgado: wetland at southern end of Pemba drainage (Pemba)	-13.089139	40.544583	A2
17104	LR594067	LR594118	LR594171	LR594231	LR594281	LR594336	Mozambique: Cabo Delgado: Rio Diquide	-11.884827	40.460208	A2
9891	LR594068	n/a	LR594172	LR594232	LR594282 LR594283	LR594337	Mozambique: Sofala province, along road 428	-20.923567	34.6662	A1

Appendix 1 (continued). Studied material of *Pelusios sinuatus* and outgroups and European Nucleotide Archive (ENA) accession numbers. Accession numbers starting with LR correspond to sequences produced for the present study.

Sample	12S	cyt <i>b</i>	ND4	Cmos	R35	Rag2	Provenance	Latitude	Longitude	mtDNA subclade
11100	LR594069	n/a	LR594173	LR594233	LR594284	LR594338	Mozambique: Sofala province, along road 428	-21.007317	34.539433	A1
6956	LR594070	LR594119	LR594174	LR594234	LR594285 LR594286	LR594339	Mozambique: Sofala: NE of Rio Save Game Reserve	-20.933333	34.316667	A1
6959	LR594071	LR594120	LR594175	LR594235	LR594287	LR594340	Mozambique: Sofala: NE of Rio Save Game Reserve	-20.7425	34.586567	A1
5215	LR594072	n/a	LR594176	LR594236	LR594288	LR594341	Mozambique: Zambesia: Moebase	-17.059513	38.699233	A2
17003	LR594073	LR594121	LR594177	LR594237	LR594289	LR594342	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve	-28.05752	32.29332	B2
17004	LR594074	LR594122	LR594178	LR594238	LR594290	LR594343	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve	-28.05752	32.29332	B2
17005	LR594075	LR594123	LR594179	LR594239	LR594291	LR594344	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve	-28.05752	32.29332	B2
17006	LR594076	LR594124	LR594180	LR594240	LR594292	LR594345	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve	-28.05752	32.29332	B2
17010	LR594077	LR594125	LR594181	n/a	n/a	n/a	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve	-28.145899	31.591881	B2
9143	LR594078	LR594126	LR594182	LR594241	LR594293	LR594346	South Africa: KwaZulu-Natal: Bonamanzi Game Reserve, Waterlily Dam	-28.05809	32.29412	B2
14040	LR594079	LR594127	LR594183	LR594242	LR594294	LR594347	South Africa: KwaZulu-Natal: Jozini, Kwa Nyamazane Conservancy	-27.391656	32.139994	B2
16199	LR594080	LR594128	LR594184	LR594243	LR594295	LR594348	South Africa: KwaZulu-Natal: Manyiseni region in Lebombo Mountains, near Mabona	-26.876389	32.011389	B2
16200	LR594081	LR594129	LR594185	LR594244	LR594296	LR594349	South Africa: KwaZulu-Natal: Manyiseni region in Lebombo Mountains, near Mabona	-26.876389	32.011389	B2
16202	LR594082	LR594130	LR594186	LR594245	LR594297	LR594350	South Africa: KwaZulu-Natal: Manyiseni region in Lebombo Mountains, near Mabona	-26.876389	32.011389	B2
16203	LR594083	LR594131	LR594187	LR594246	LR594298	LR594351	South Africa: KwaZulu-Natal: Manyiseni region in Lebombo Mountains, near Mabona	-26.876389	32.011389	B2
10614	LR594084	n/a	LR594188	LR594247	LR594299	LR594352	South Africa: KwaZulu-Natal: Ndumo Game Reserve	-26.885692	32.223672	B2
10615	LR594085	LR594132	LR594189	LR594248	LR594300	LR594353	South Africa: KwaZulu-Natal: Ndumo Game Reserve	-26.874944	32.231997	B2
14041	LR594086	LR594133	LR594190	LR594249	LR594301	LR594354	South Africa: KwaZulu-Natal: Ndumo Game Reserve	-26.891275	32.299	B2

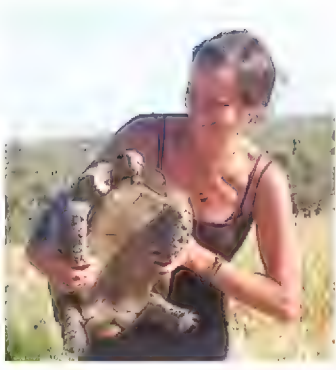
Phylogeography of *Pelusios sinuatus* in East Africa

Appendix 1 (continued). Studied material of *Pelusios sinuatus* and outgroups and European Nucleotide Archive (ENA) accession numbers. Accession numbers starting with LR correspond to sequences produced for the present study.

Sample	12S	cyt <i>b</i>	ND4	Cmos	R35	Rag2	Provenance	Latitude	Longitude	mtDNA subclade
13590	LR594087	LR594134	LR594191	LR594250	LR594302	LR594355	South Africa: KwaZulu-Natal: Ndumo Game Reserve: Mabayani	-26.865118	32.240964	B2
5216	FR716876	FR716937	FR716985	FR717029	FR717077	FR717122	South Africa: KwaZulu-Natal: Phinda Game Reserve	-27.843744	32.335521	B2
5217	FR716877	FR716938	FR716986	FR717030	FR717078	FR717123	South Africa: KwaZulu-Natal: Phinda Game Reserve	-27.843744	32.335521	B2
17014	LR594088	LR594135	LR594192	LR594251	LR594303	LR594356	South Africa: KwaZulu-Natal: St. Lucia: Crocodile Centre	-28.357158	32.419512	B2
17015	LR594089	LR594136	LR594193	LR594252	LR594304	LR594357	South Africa: KwaZulu-Natal: St. Lucia: Crocodile Centre	-28.357158	32.419512	B2
17016	LR594090	LR594137	LR594194	LR594253	LR594305	LR594358	South Africa: KwaZulu-Natal: St. Lucia: Crocodile Centre	-28.357158	32.419512	B2
17028	LR594091	LR594138	LR594195	LR594254	LR594306	LR594359	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17029	LR594092	LR594139	LR594196	LR594255	LR594307	LR594360	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17030	LR594093	LR594140	LR594197	LR594256	LR594308	LR594361	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17031	LR594094	LR594141	LR594198	LR594257	LR594309	LR594362	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17038	LR594095	LR594142	LR594199	LR594258	LR594310	LR594363	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B1
17039	LR594096	LR594143	LR594200	LR594259	LR594311	LR594364	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17040	LR594097	LR594144	LR594201	LR594260	LR594312	LR594365	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17041	LR594098	LR594145	LR594202	LR594261	LR594313	LR594366	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B2
17042	LR594099	LR594146	LR594203	LR594262	LR594314	LR594367	South Africa: Limpopo: Hoedspruit: Bush Pub Inn	-24.35039	31.152019	B1
17061	LR594100	LR594147	LR594204	LR594263	LR594315	LR594368	South Africa: Limpopo: Lapalala	-23.89392	28.29516	B1
17068	LR594101	LR594148	LR594205	LR594264	LR594316	LR594369	South Africa: Limpopo: Lapalala	-23.89392	28.29516	B1
16206	LR594102	LR594149	LR594206	LR594265	LR594317	LR594370	South Africa: Limpopo: Palabora Mining Company, near Loolo Dam, S of Phalaborwa	-24.018889	31.140833	B1
16197	LR594103	LR594150	LR594207	LR594266	LR594318	LR594371	South Africa: Limpopo: Palabora Mining Company, SE of Phalaborwa	-24	31.210556	B1
16196	LR594104	LR594151	LR594208	n/a	LR594319	LR594372	South Africa: Limpopo: Palabora Mining Company: Cleveland Nature Reserve, in Olifants River near picnic site	-24.03056	31.19306	B2
17073	LR594105	LR594152	LR594209	LR594267	LR594320	LR594373	South Africa: Limpopo: Vaalwater	-23.98716	28.37323	B1

Appendix 1 (continued). Studied material of *Pelusios sinuatus* and outgroups and European Nucleotide Archive (ENA) accession numbers. Accession numbers starting with LR correspond to sequences produced for the present study.

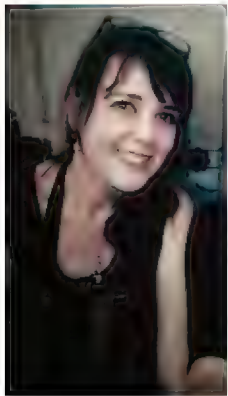
Sample	12S	cyt <i>b</i>	ND4	Cmos	R35	Rag2	Provenance	Latitude	Longitude	mtDNA subclade
13585	LR594106	LR594153	LR594210	LR594268	LR594321	LR594374	South Africa: Mpumalanga: Kruger National Park: Shingwedzi River near Shingwedzi Camp	-23.107365	31.439066	B1
13586	LR594107	n/a	LR594211	n/a	n/a	n/a	South Africa: Mpumalanga: Kruger National Park: Shingwedzi River near Shingwedzi Camp	-23.107365	31.439066	B1
16214	LR594108	LR594154	LR594212	LR594269	LR594322	LR594375	Tanzania: Kilimanjaro Region: Moshi	-3.34261	37.319831	A3
16269	LR594109	LR594155	LR594213	LR594270	LR594323	LR594376	Tanzania: Manyara Region: Kikuletwa Hotsprings	-3.443532	37.193393	A3
16270	LR594110	LR594156	LR594214	LR594271	LR594324	LR594377	Tanzania: Manyara Region: Kikuletwa Hotsprings	-3.443532	37.193393	A3
	Outgroups									
	<i>Pelusios marani</i>									
5214	FR716869	FR716928	FR716979	n/a	n/a	n/a				
	<i>Pelomedusa variabilis</i>									
—	AF039066	AF039066	AF039066	n/a	n/a	n/a				



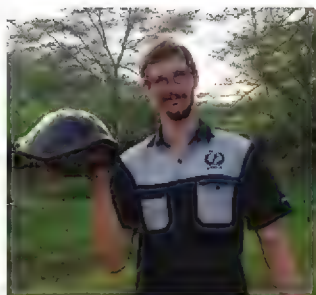
Melita Vamberger is a Slovenian herpetologist and evolutionary biologist working at the Senckenberg Natural History Collections, Dresden, Germany. Melita studied Biology at the University of Ljubljana, Slovenia, focusing on the natural history of the European Pond Turtle (*Emys orbicularis*). After her diploma, Melita moved to Germany for her Ph.D. at the University of Leipzig, studying the phylogeography and hybridization of two closely related freshwater turtle species (*Mauremys caspica* and *M. rivulata*). Melita's main interests are speciation, gene flow, adaptation, and evolution of different turtle taxa using an integrative approach that combines genetic and ecological methods, especially in the Western Palearctic and sub-Saharan Africa.



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Mind the gap—Is the distribution range of *Pelomedusa galeata* really disjunct in western South Africa?

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Abstract.—Records from the putative gap in the distribution range of *Pelomedusa galeata* in western South Africa provide evidence for the occurrence of helmeted terrapins in those areas. Further research is needed to reveal the genetic and taxonomic identity of these populations.

Keywords. helmeted terrapin, Northern Cape Province, Pelomedusidae, Testudines, turtle

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Helmeted terrapins have a wide distribution across sub-Saharan Africa and the southwestern Arabian Peninsula. Together with their sister taxon, the African hinged terrapins (*Pelusios*), helmeted terrapins constitute the side-necked turtle family Pelomedusidae (TTWG 2017). While it was assumed for decades that all helmeted terrapins are conspecific (Boycott and Bourquin 2008; Branch 2008; Ernst and Barbour 1989; Wermuth and Mertens 1961, 1977), several investigations revealed deep genetic divergences between geographically coherent populations, which resemble or exceed the divergences of distinct *Pelusios* species (Fritz et al. 2011, 2014; Petzold et al. 2014; Vamberger et al. 2018; Vargas-Ramírez et al. 2010; Wong et al. 2010). This resulted in the formal recognition of no less than 10 distinct species (Petzold et al. 2014; TTWG 2017; Vamberger et al. 2018). In addition to these, a minimum of five unnamed species are thought to exist, which are characterized by similar genetic divergences but otherwise only insufficiently known (Fritz et al. 2015; Nagy et al. 2015; Petzold et al. 2014; Vamberger et al. 2018; Vargas-Ramírez et al. 2016).

In the Southern African region south of the Cunene and Zambezi Rivers, two species are known to occur, the South African Helmeted Terrapin *Pelomedusa galeata* (Schoepff, 1792) and the Common Helmeted Terrapin *Pelomedusa subrufa* (Bonnaterre, 1789) (Fritz et al. 2015; Petzold et al. 2014; Vamberger et al. 2018). *Pelomedusa galeata* is distributed in most of South Africa and replaced in the countries north of South Africa by *P. subrufa*, which enters also northeastern South Africa (provinces of Limpopo and Mpumalanga). In these

provinces, the distribution ranges of the two species abut and the closest records of *P. galeata* and *P. subrufa* are separated only by 80 km, so that overlapping ranges seem possible (Vamberger et al. 2018). *Pelomedusa galeata* shows pronounced phylogeographic structuring, with two genetically deeply divergent groups in the east and west of South Africa that most likely represent two distinct species. The eastern group is phylogeographically structured, with three parapatric subgroups (Fritz et al. 2015; Petzold et al. 2014; Vamberger et al. 2018).

Detailed distribution maps for helmeted terrapins show a patchy range for Southern Africa, with large putative gaps in western South Africa, southern Mozambique, southern Namibia, and most of Botswana (Boycott 2014; Boycott and Bourquin 2008; TTWG 2017). Except for southern Mozambique, these regions are very arid, suggestive of pessimal conditions for a freshwater turtle like the helmeted terrapin. However, *P. subrufa* is known to cope with year-long drought. In Namibia (Omaheke), terrapins of this species may evidently survive up to six years burrowed in the soil (Petzold et al. 2014) and surface only after the rare rainfalls. Boycott and Bourquin (2008) suggested that helmeted terrapins take advantage of man-made farm dams and expanded their range into otherwise unsuitable regions, including semi-desert. However, records for *P. subrufa* from the mouths of temporary streams in the Namib Desert may well represent natural occurrences of terrapins washed downstream during the rare floods there (A. Schleicher, pers. comm.).

One large distribution gap is located in western South Africa and concerns *P. galeata*. It more or less separates the two genetically deeply divergent groups of this species (Vamberger et al. 2018). During fieldwork in October and November 2018, the first two authors

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Fig. 1. *Pelomedusa galeata* from the Ratelfontein farm near Calvinia observed directly after rainfall, 16 February 2019. For the location of the farm, see Fig. 2 (locality 1). Photos: C.A. van Niekerk.

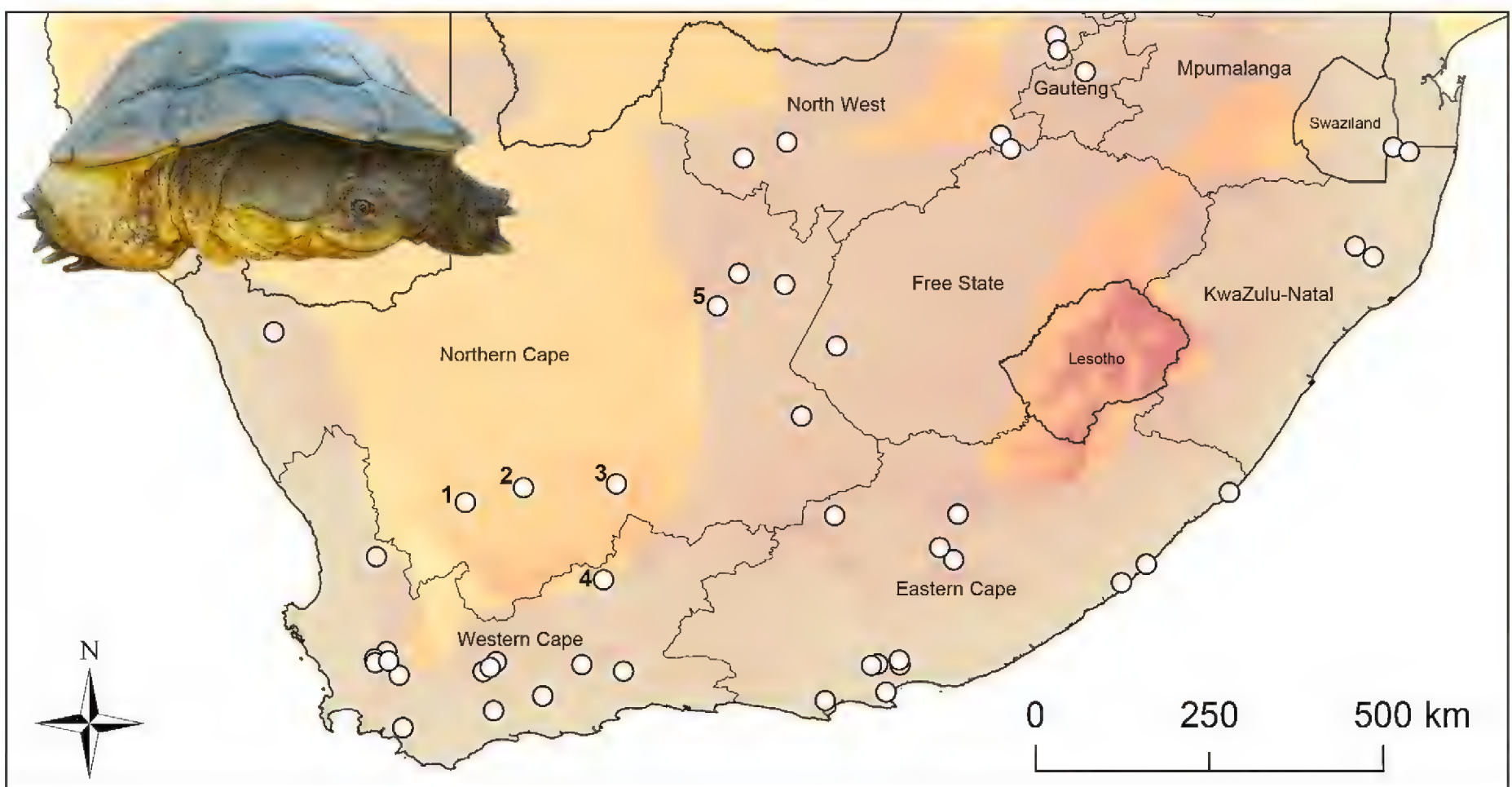


Fig. 2. Distribution range of Helmeted Terrapins (shaded in grey), with our records of *Pelomedusa galeata* in South Africa (white circles). New records of *P. galeata* in or close to the putative distribution gap: 1 – Nineteen turtles at Ratelfontein farm, near Calvinia (16 February 2019), 2 – Observations of locals at Williston, 3 – Near Carnarvon (shell, collected 25 October 2018), 4 – One terrapin near Beaufort West (4 March 2017), 5 – Two terrapins near Griekwastad (28 October 2018). Inset: *Pelomedusa galeata* from the Ratelfontein farm. Photo: C.A. van Niekerk.

had the opportunity to examine parts of this putative distribution gap for the presence of helmeted terrapins. In addition to direct observations, interviews with farmers and locals contributed further information. It is common knowledge there that helmeted terrapins are present but very scarce. They are seen only after the rare rainfall events, when the terrapins are walking to waterholes (Fig. 1). Together with our records substantiated by specimens,

this provides the first evidence for the occurrence of *P. galeata* in the central part of the Northern Cape Province of South Africa (Fig. 2). We assume that the helmeted terrapin has an even wider distribution and also occurs northwards to Namibia, and that a distribution gap does not exist at all. *Pelomedusa* are very elusive animals in arid regions, and we presume that the putative gap reflects not a real absence of helmeted terrapins but

a lack of records. Further research is needed to reveal which genetic lineage of *P. galeata* occurs in this area and whether there is a contact zone of the two lineages currently identified with *P. galeata*.

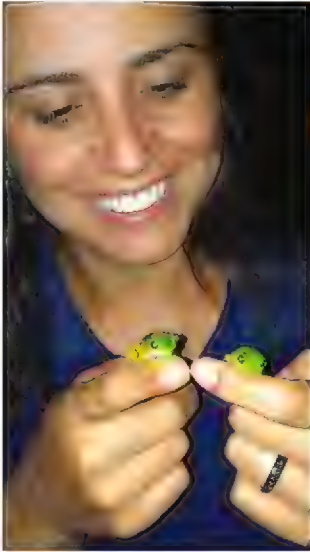
Acknowledgements.—We would like to thank C.A. and Berno van Niekerk from Ratelfontein farm, Adam van Greunen, Isak Dreyer, Pieter and Elmarie Naude from Die Ark, Rodney Bartholomew, Haas van Niekerk, Bertus and Roux Steenkamp, Nadia and Abri van Zyl, Adriaan Jordaan, Nick Telford, and Krystal Tolley for all the valuable information, pictures, and help during fieldwork. Fieldwork of M.V. and P.R.A. was supported by the DGHT (Deutsche Gesellschaft für Herpetologie und Terrarienkunde) and the BCG (British Chelonian Group). M.D.H. was supported by the Mapula Trust. Fieldwork was permitted by the North West Province (NW 6124/10/2018) and Northern Cape Province (0729/2018 and 0730/2018).

Literature Cited

- Boycott RC. 2014. *Pelomedusa subrufa* (Bonnaterre, 1789). Pp. 54 In: *Atlas and Red List of the Reptiles of South Africa, Lesotho and Swaziland*. Suricata 1. Editors, Bates MF, Branch WR, Bauer AM, Burger M, Marais J, Alexander GJ, de Villiers MS. South African National Biodiversity Institute (SANBI) Publishing, Pretoria, South Africa. xviii, 486 p.
- Boycott RC, Bourquin O. 2008. *Pelomedusa subrufa* (Lacépède 1788) – helmeted turtle, helmeted terrapin. Pp. 007.1–007.6 In: *Conservation Biology of Freshwater Turtles and Tortoises: a Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group*. Chelonian Research Monographs 5. Editors, Rhodin AGJ, Pritchard PCH, van Dijk PP, Saumure RA, Buhlmann KA, Iverson JB. Chelonian Research Foundation, Lunenburg, Massachusetts, USA. Loose-leaf (pagination variable).
- Branch WR. 2008. *Tortoises, Terrapins & Turtles of Africa*. Struik, Cape Town, South Africa. 128 p.
- Ernst CH, Barbour RW. 1989. *Turtles of the World*. Smithsonian Institution Press, Washington, D.C., USA, and London, United Kingdom. xii, 313 p.
- Fritz U, Branch WR, Hofmeyr MD, Maran J, Prokop H, Schleicher A, Široký P, Stuckas H, Vargas-Ramírez M, Vences M, et al. 2011. Molecular phylogeny of African hinged and helmeted terrapins (Testudines: Pelomedusidae: *Pelusios* and *Pelomedusa*). *Zoologica Scripta* 40: 115–125.
- Fritz U, Kehlmaier C, Mazuch T, Hofmeyr MD, du Preez L, Vamberger M, Vörös J. 2015. Important new records of *Pelomedusa* species for South Africa and Ethiopia. *Vertebrate Zoology* 65: 383–389.
- Fritz U, Petzold A, Kehlmaier C, Kindler C, Campbell P, Hofmeyr MD, Branch WR. 2014. Disentangling the *Pelomedusa* complex using type specimens and historical DNA (Testudines: Pelomedusidae). *Zootaxa* 3795: 501–522.
- Nagy ZT, Kielgast J, Moosig M, Vamberger M, Fritz U. 2015. Another candidate species of *Pelomedusa* (Testudines: Pelomedusidae) from the Democratic Republic of the Congo? *Salamandra* 51: 212–214.
- Petzold A, Vargas-Ramírez M, Kehlmaier C, Vamberger M, Branch WR, du Preez L, Hofmeyr MD, Meyer L, Schleicher A, Široký P, et al. 2014. A revision of African helmeted terrapins (Testudines: Pelomedusidae: *Pelomedusa*), with descriptions of six new species. *Zootaxa* 3795: 523–548.
- TTWG (Turtle Taxonomy Working Group), Rhodin AGJ, Iverson JB, Bour R, Fritz U, Georges A, Shaffer HB, van Dijk PP. 2017. *Turtles of the World. Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status*. 8th edition. Chelonian Research Monographs 7. Chelonian Research Foundation, Lunenburg, Massachusetts, USA. 292 p.
- Vamberger M, Hofmeyr MD, Ihlow F, Fritz U. 2018. In quest of contact: phylogeography of helmeted terrapins (*Pelomedusa galeata*, *P. subrufa* sensu stricto). *PeerJ* 6: e4901.
- Vargas-Ramírez M, Petzold A, Fritz U. 2016. Distribution modelling and conservation assessment for helmeted terrapins (*Pelomedusa* spp.). *Salamandra* 52: 306–316.
- Vargas-Ramírez M, Vences M, Branch WR, Daniels SR, Glaw F, Hofmeyr MD, Kuchling G, Maran J, Papenfuss T, Široký P, et al. 2010. Deep genealogical lineages in the widely distributed African Helmeted Terrapin: evidence from mitochondrial and nuclear DNA (Testudines: Pelomedusidae: *Pelomedusa subrufa*). *Molecular Phylogenetics and Evolution* 56: 428–440.
- Wermuth H, Mertens R. 1961. *Schildkröten, Krokodile, Brückenechsen*. VEB Gustav Fischer Verlag, Jena, Germany. XXVI, 422 p.
- Wermuth H, Mertens R. 1977. Testudines, Crocodylia, Rhynchocephalia. *Das Tierreich* 100: 1–174.
- Wong RA, Fong JJ, Papenfuss TJ. 2010. Phylogeography of the African Helmeted Terrapin, *Pelomedusa subrufa*: genetic structure, dispersal, and human introduction. *Proceedings of the California Academy of Sciences* 61: 575–585.



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Margaretha D. Hofmeyr is Professor Emerita at the Biodiversity and Conservation Biology Department, University of the Western Cape, South Africa. She is an ecophysiologist by training and first studied large ungulates before switching to chelonians. Her ecophysiological studies revealed that South African tortoises have many unique characteristics, which stimulated her interest in their genetic diversity and systematics. Margaretha has published extensively on the ecology and phylogeography of sub-Saharan tortoises and turtles, and she is closely involved in conservation projects that focus on threatened tortoises. This work resulted in her being awarded the 2015 Sabin Turtle Conservation Prize. Margaretha is a member, and Regional Vice-Chair for Africa, of the IUCN/SSC TFTSG and she coordinated the 2014 and 2018 Red List Assessments for South African tortoises and freshwater turtles.



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Geographic range extension of Speke's Hinge-back Tortoise *Kinixys spekii* Gray, 1863

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Abstract.—*Kinixys spekii* has a wide distribution range across sub-Saharan Africa, having been reported from Angola, Botswana, Burundi, the Democratic Republic of the Congo, eSwatini, Kenya, Malawi, Mozambique, Namibia, South Africa, Tanzania, Zambia, and Zimbabwe. *Kinixys spekii* inhabits savannah and dry bushveld habitats and was previously considered an inland species. However, recent records suggest a more extensive geographical distribution. Here, we provide genetically verified records for Angola, South Africa, and Mozambique, and discuss reliable sightings for Rwanda. These new records extend the range significantly to the east and west, and provide evidence for the occurrence of this species along the coast of the Indian Ocean in South Africa and Mozambique.

Keywords. Africa, Angola, chelonians, distribution, Mozambique, Reptilia, Rwanda, Testudinidae

Citation: Ihlow F, Farooq HM, Gvoždík V, Hofmeyr MD, Conradie W, Campbell PD, Harvey J, Verburgt L, Fritz U. 2019. Geographic range extension of Speke's Hinge-back Tortoise *Kinixys spekii* Gray, 1863. *Amphibian & Reptile Conservation* 13(2) [Special Section]: 61–67 (e195).

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The genus *Kinixys* currently comprises eight species (Kindler et al. 2012; TTWG 2017): *K. belliana* Gray, 1830; *K. erosa* (Schweigger, 1812); *K. homeana* Bell, 1827; *K. lobatsiana* Power, 1927; *K. natalensis* Hewitt, 1935; *K. nogueyi* (Lataste, 1886); *K. spekii* Gray, 1863; and *K. zombensis* Hewitt, 1931. Two of these species are confined to rainforest habitats (*K. erosa*, *K. homeana*), while one is restricted to north-western Africa (*K. nogueyi*), and the remaining five occupy savannah and forest habitats in eastern and south-eastern Africa.

Speke's Hinge-back Tortoise, *Kinixys spekii*, has an extensive geographical distribution range, spanning twelve countries, from southern Kenya southward to eSwatini (formerly Swaziland), southern Mozambique, and north-eastern South Africa, where it reaches its southernmost limit (Boycott and Bourquin 2000; Branch et al. 1995; Broadley 1989a; Spawls et al. 2004, 2018; TTWG 2017). The species' westward range extends across Zimbabwe, Zambia, and northern Botswana

into the Zambezi (formerly Caprivi) region of Namibia (Broadley 1989a, 1993; Jacobsen et al. 1986; Pienaar et al. 1983; TTWG 2017). According to Broadley (1989a,b, 1993), *K. spekii* is confined to the inland parts of southern and central Africa, inhabiting the eastern plateau slopes, while the range of *K. zombensis* extends along the East African coastal plain from Kenya to the KwaZulu-Natal Province of South Africa. However, a few records from Kenya (Watamu, 3.34250°S, 40.02740°E; in the vicinity of Kilifi, voucher specimen in the collection of the Yale Peabody Museum of Natural History YPM HERR 014516) suggest that the range of *K. spekii* reaches the northern coastal areas as well. Since some photographs of tortoises from Watamu shown in Spawls et al. (2004, 2018) morphologically resemble *K. zombensis* rather than *K. spekii*, this record requires verification.

In terms of habitat, *K. spekii* has been recorded from savannah, tropical bushveld, tropical savannah, sour bushveld, and the thornveld of the Lebombo Plateau

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(Boycott 2001; Boycott and Bourquin 2000; Branch 2008). According to Broadley (1989a), this species prefers moist savannah woodlands, such as Miombo and Mopane (woodlands dominated by *Brachystegia* and *Colophospermum* species, respectively), but also occurs in drier deciduous woodlands and thickets dominated by *Vachellia* (until recently *Acacia*; Kyalangalilwa et al. 2013) and *Commiphora* in the north-eastern part of its range (Broadley 1989a).

For the present contribution, records for *K. spekii* were compiled from the scientific literature and museum collections, and supplemented with a few selected sightings from the online Virtual Museum Database iNaturalist (<https://www.inaturalist.org>) to discuss the distribution range of *K. spekii*. Several new and genetically verified records from Angola, South Africa, and coastal Mozambique are also presented, which extend the species' known distribution range considerably. Genetic verification relied on an mtDNA sequence coding for the partial NADH dehydrogenase subunit 4 (ND4) and adjacent tRNAs.

Unfortunately, online databases and data aggregators are often compromised by incorrect identifications and outdated taxonomy, and either provide no photographic vouchers or ones that are unsuitable for facilitating verification before using the data. Nevertheless, two photographic vouchers deposited with iNaturalist (<https://www.inaturalist.org/observations/18255494>, <https://www.inaturalist.org/observations/1047117>) could clearly be assigned to *K. spekii*, based on characteristic coloration patterns and shell shape. These records provide evidence that the species occurs as far north-west as Nyagatare (1.42321°S, 30.63027°E) and Akagera (1.55162°S, 30.60760°E) in Rwanda, which is in accordance with Spawls et al. (2004, 2018), who reported isolated records for *K. spekii* from Akagera, the Ruzizi Plain, and the southern Kerio Valley in eastern Rwanda. To the east, two genetically verified *K. spekii* (Museum of Zoology, Senckenberg Dresden, Tissue Collection: MTD 17106, 17107; European Nucleotide Archive accession numbers: LR723010, LR723011) were sampled and released by Luke Verburgt in February 2014 on the Afungi Peninsula, Cabo Delgado Province, coastal Mozambique (10.81939°S, 40.54842°E; but see below). In addition, four genetically verified adult *K. spekii* (MTD 20463, 20464; LR723016, LR723017) were sampled and released by Harith M. Farooq within a 10 km radius of the Lúrio University in Pemba, Cabo Delgado Province, Mozambique (12.97540°S, 40.57083°E) in 2014. An adult female (12.97615°S, 40.10205°E) and an adult male tortoise (12.99333°S, 39.94861°E) were sampled and released by William R. Branch in March 2017 in Ancuabe, Cabo Delgado Province, Mozambique, and both were genetically verified to represent *K. spekii* (MTD 20463-20464; LR723016-7). In the west, a genetic sample (SANBI 2126; LR723018) collected by Thomas Branch in October 2008 shows that *K. spekii* also occurs

near Saurimo, Lunda Sul Province, Angola (9.39694°S, 20.43194°E).

With the exception of the tortoises from the Afungi Peninsula, all specimens could unambiguously be identified morphologically as *K. spekii* based on the following characteristics: beak unicuspid, carapace with well-developed hinge, carapace distinctly depressed, and posterior marginal scutes not recurved or serrated. Previous records for Mozambique were limited to the southwest (Boycott and Bourquin 2000), the vicinity of Ressano Garcia, southern Mozambique (Broadley 1989b), and the Maputo Elephant Reserve situated along the coast of southern Mozambique (voucher specimen in the collection of the Ditsong National Museum of Natural History TM 41761; Broadley 1993; Fig. 1). To the south, samples collected in the KwaZulu-Natal Province, South Africa, were genetically verified as *K. spekii* (MTD 13594 from Mkhuze Game Reserve; LR723019; MTD 7457 from the vicinity of Mtubatuba; HE662316). Previously, *K. spekii* was only known from the extreme northern border of the province, adjacent to eSwatini and Mozambique (Bourquin 2004; Boycott 2014).

The abovementioned records enlarge the known geographical distribution range of *K. spekii*, but also demonstrate that the species' distribution range is still incompletely known. Unfortunately, morphological traits overlap between *Kinixys* species, making species identification in potential zones of sympatry extremely difficult. For instance, *K. spekii* co-occurs with *K. zombensis* in the Maputo Elephant Reserve in Mozambique, and the Ndumo Game Reserve in South Africa. In the Waterberg area of South Africa *K. spekii* is found close to *K. lobatsiana* populations, and it was recorded together with *K. natalensis* in the vicinity of Jameson's Drift, in the Lebombo Mountains in KwaZulu-Natal as well as in the area of Hoedspruit. In these areas, hybridization is possible, which further complicates identification. For example, the two tortoises collected from the Afungi Peninsula in northern Mozambique morphologically resemble *K. zombensis* (Fig. 3; domed carapace, radial coloration pattern), but their mtDNA sequences match those of *K. spekii*, suggesting hybridization. Additional genetic studies using nuclear genes are required to verify their putative hybrid status. However, if there is no evidence for a hybrid identity in these tortoises, then the morphological characters thought to be diagnostic for discerning *K. spekii* and *K. zombensis* would be seriously challenged. Moreover, the characteristic coloration patterns commonly used to distinguish between *Kinixys* species tend to fade with age, rendering older tortoises more or less uniformly colored (Branch 2008; Broadley 1993; Fig. 2). Hence, hinge-back tortoises are frequently misidentified. Genetic verification of specimens which were morphologically determined by renowned African herpetologists revealed misidentification rates ranging from 2% for *K. zombensis* to 66% for *K. natalensis* (F. Ihlow and U. Fritz unpub.

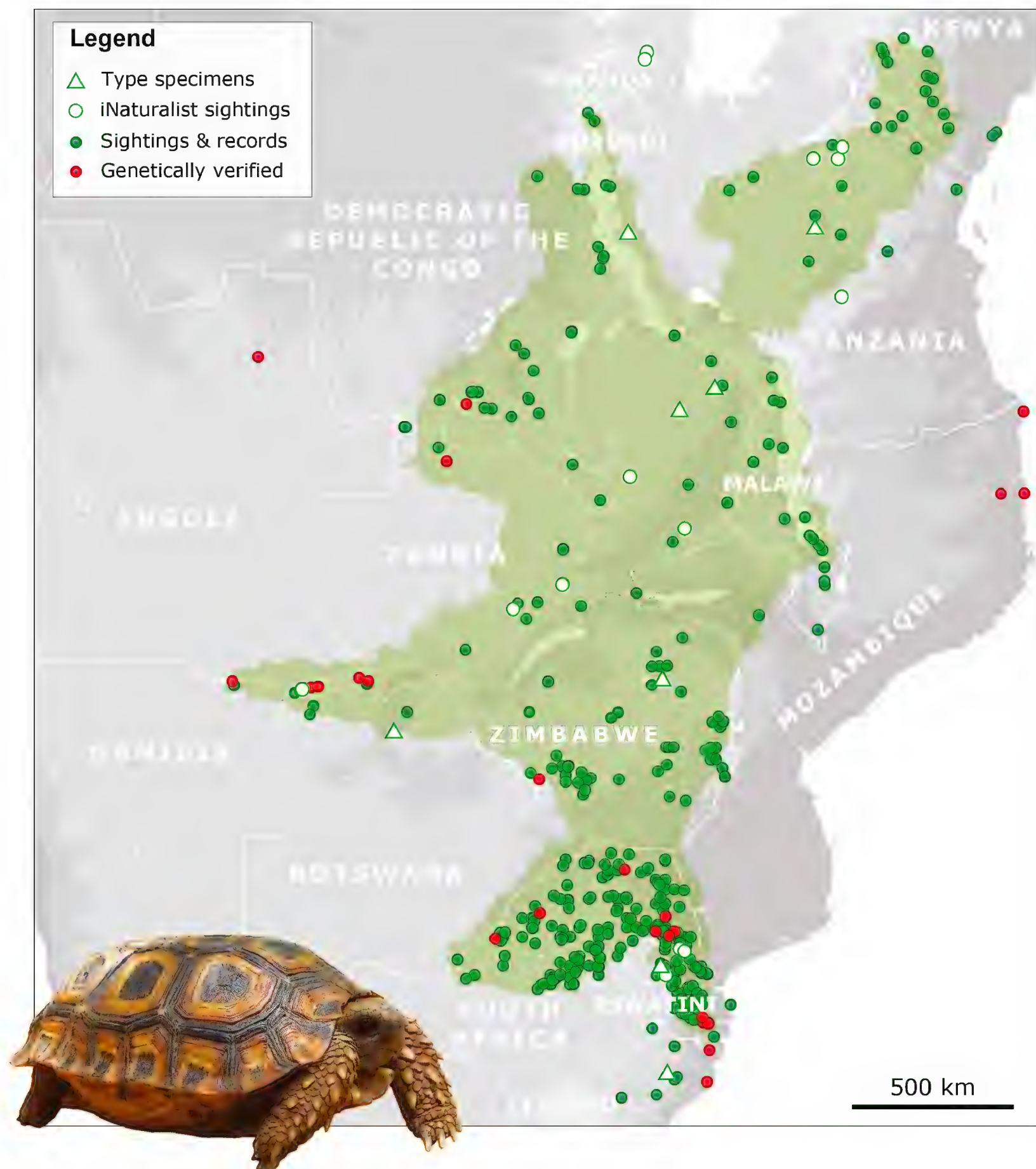


Fig. 1. Known distribution of *Kinixys spekii*. Range according to TTWG (2017) is displayed as green shaded area. Open circles refer to iNaturalist observations, while solid green circles represent literature records and specimens deposited in scientific collections. Solid red circles correspond to genetically verified records, and triangles to name-bearing type specimens of *Kinixys spekii* and its synonyms.

data). While older, uniformly colored *K. lobatsiana* are mainly confused with *K. spekii* and vice versa, young *K. spekii* are frequently confused with *K. natalensis* (Fig. 2). The most reliable morphological trait allowing distinction between *K. natalensis* and young *K. spekii* is the tricuspid beak of *K. natalensis*, whereas *K. spekii* has a unicuspid beak. Old uniformly colored *K. lobatsiana* can be distinguished from *K. spekii* based on the posterior shell rim, which is serrated in *K. lobatsiana* and smooth in *K. spekii* (Fig. 2).

The high misidentification rates show that the established morphological characters for species determination are insufficient and call into question the reliability of published records, photographic vouchers,

and even the identification of specimens (including name-bearing type specimens) housed in scientific collections. Given that the putative distribution ranges (TTWG 2017), which represent an essential tool for the conservation and management of these species, also largely rely on collection databases and morphologically identified individuals, these should be treated with caution.

To ensure correct species identification of challenging specimens, molecular genetic verification is strongly recommended until more robust morphological characters have been revealed. Photographic vouchers should include dorsal, ventral, and lateral views to facilitate accurate species identifications. For *K.*



Fig. 2. **Top:** Lateral views of adult *Kinixys lobatsiana* (left) and *K. spekii* (right). **Center:** Ventral views of young (SCL 106 mm) and adult (SCL 161 mm) *K. lobatsiana* (left) and young (SCL 131 mm) and adult (SCL 151 mm) *K. spekii* (right). Note the strongly serrated posterior marginal scutes in *K. lobatsiana* compared to the smooth carapace rim in *K. spekii*. **Bottom:** Lateral views of adult *K. natalensis* (left) and young *K. spekii* (right). Photos: James Harvey and Flora Ihlow.

natalensis additional voucher photographs showing the tricuspid beak should be taken.

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Fig. 3. Top: Lateral views of the putative hybrids from the Afungi Peninsula, Cabo Delgado Province, Mozambique, which have mtDNA sequences of *Kinixys spekii* but morphologically resemble *K. zombensis*. **Bottom:** Lateral views of genetically verified *K. zombensis* from KwaZulu-Natal Province, South Africa. Photos: Luke Verburgt and Flora Ihlow.

Literature Cited

- Bourquin O. 2004. Reptiles (Reptilia) in KwaZulu-Natal: 1 – Diversity and distribution. *Durban Museum Novitates* 29: 57–103.
- Boycott RC, Bourquin O. 2000. *The Southern African Tortoise Book — A Guide to Southern African Tortoises, Terrapins and Turtles*. Revised Edition. Southern Book Publishers, Johannesburg, South Africa. 228 p.
- Boycott RC. 2001. The terrapins and tortoises (Chelonia: Pelomedusidae and Testudinidae) of Swaziland. *Durban Museum Novitates* 26: 25–37.
- Boycott RC. 2014. *Kinixys spekii* Gray, 1863, Speke's hinged-back tortoise. Pp. 78 In: *Atlas and Red List of the Reptiles of South Africa, Lesotho and Swaziland*. Editors, Bates MF, Branch WR, Bauer AM, Burger M, Marais J, Alexander GJ, de Villiers M. South African National Biodiversity Institute, Pretoria, South Africa. 485 p.
- Branch WR, Benn GA, Lombard AT. 1995. The tortoises (Testudinidae) and terrapins (Pelomedusidae) of southern Africa: their diversity, distribution and conservation. *South African Journal of Zoology* 30: 91–102.
- Branch B. 2008. *Tortoises, Terrapins and Turtles of Africa*. Struik, Cape Town, South Africa. 129 p.
- Broadley DG. 1989a. *Kinixys spekii*, Speke's hinged tortoise. Pp. 52–54 In: *The Conservation Biology of Tortoises*. Editors, Swingland IR, Klemens MW. IUCN, Gland, Switzerland. 203 p.
- Broadley DG. 1989b. *Kinixys belliana*, Bell's hinged tortoise. Pp. 49–52 In: *The Conservation Biology of Tortoises*. Editors, Swingland IR, Klemens MW. IUCN, Gland, Switzerland. 203 p.
- Broadley DG. 1993. A review of the southern African species of *Kinixys* Bell (Reptilia: Testudinidae). *Annals of the Transvaal Museum* 36: 41–52.
- Jacobsen NHG, Newbery RE, Petersen WL. 1986. *A Checklist of the Herpetofauna of the Transvaal Provincial Nature Reserves*. Transvaal Nature Conservation Division, Pretoria, South Africa. 38 p.
- Kindler C, Branch WR, Hofmeyr MD, Maran J, Šíroký P, Vences M, Harvey J, Hauswaldt JS, Schleicher A, Stuckas H et al. 2012. Molecular phylogeny of African hinge-back tortoises (*Kinixys*): implications for phylogeography and taxonomy (Testudines: Testudinidae). *Journal of Zoological Systematics and Evolutionary Research* 50: 192–201.
- Kyalangalilwa B, Boatwright JS, Daru BH, Maurin O, van der Bank M. 2013. Phylogenetic position and revised classification of *Acacia* s.l. (Fabaceae: Africa. Struik, Cape Town, South Africa. 129 p.

Mimosoideae) in Africa, including new combinations in *Vachellia* and *Senegalia*. *Botanical Journal of the Linnean Society* 172: 500–523.

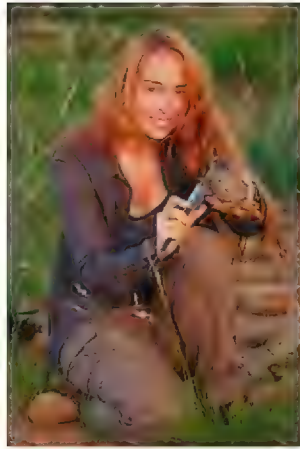
Pienaar U de V, Haacke WO, Jacobsen NHG. 1983. *The Reptiles of the Kruger National Park*. National Parks Board South Africa, Pretoria, South Africa. 236 p.

Spawls S, Howell K, Drewes C, Ashe J. 2004. *A Field Guide to the Reptiles of East Africa*. Gardners Books, Eastbourne, United Kingdom. 544 p.

Spawls S, Howell K, Hinkel H, Menegon M. 2018.

Field Guide to East African Reptiles. Bloomsbury Publishing PLC, London, United Kingdom. 624 p.

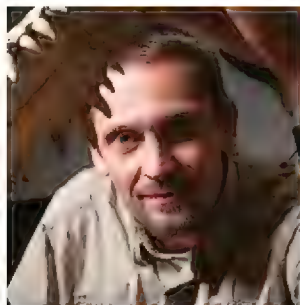
TTWG [Turtle Taxonomy Working Group: Rhodin AGJ, Iverson JB, Bour R, Fritz U, Georges A, Shaffer HB, van Dijk PP]. 2017. *Turtles of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status*. 8th Edition. Chelonian Research Monographs 7. Chelonian Research Foundation, Lunenburg, Massachusetts, USA and Turtle Conservancy, Ojai, California, USA. 292 p.



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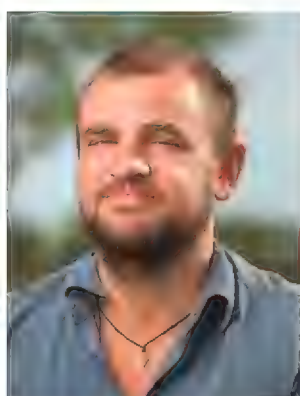
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Werner Conradie holds a Masters in Environmental Science (M. Env. Sc.) and has 12 years of experience with the southern African herpetofauna, with his main research interests focusing on the taxonomy, conservation, and ecology of amphibians and reptiles. Werner has published numerous principal and collaborative scientific papers, and has served on a number of conservation and scientific panels, including the Southern African Reptile and Amphibian Relisting Committees. He has undertaken research expeditions to many African countries including Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Zambia, and Zimbabwe. Werner is currently the Curator of Herpetology at the Port Elizabeth Museum (Bayworld), South Africa.



Patrick D. Campbell holds a B.Sc. in Biological Sciences with 33 years working experience in the department of Life Sciences (Zoology) at the Natural History Museum, London, NHM (UK). Patrick has travelled the world on official duty as collector, diver, science officer, surveyor, and speaker at various conferences, as far afield as China, Brazil, Thailand, Kenya, French Guiana, Ecuador, the United States, Spain, and Milos to name but a few. He has published nearly 50, mostly collaborative, papers on a variety of topics involving a number of different lower vertebrate species but most recently, and primarily, on the taxonomy, osteology, and conservation of reptiles. Patrick is currently the Senior Curator of Reptiles at the NHM (UK).



James Harvey works as an independent herpetologist, ecological researcher, and consultant, living in South Africa. He holds degrees in Zoology, Hydrology, and Environmental Management, and has 16 years' experience working with faunal biodiversity. James has performed ecological fieldwork widely, primarily within Africa, in such countries as South Africa, Botswana, Zimbabwe, Angola, Malawi, Mozambique, Kenya, Mali, Madagascar, Vietnam, and the Democratic Republic of the Congo. His interests are diverse but center on the taxonomy, ecology, and conservation of herpetofauna and other biodiverse groups. James has contributed to conservation assessments, workshops, and Red Data publications on reptiles, amphibians, mammals, and plants for the southern and eastern African regions. He regularly attends herpetological conferences, and has published numerous scientific papers and was a contributing author on many more.



Luke Verburgt is a specialist consulting herpetologist working throughout Africa, with his professional and scientific research experience extending over 16 years. Luke has published 18 internationally recognized scientific papers to date on topics including herpetology, evolutionary biology, ecological physiology, and animal behavior. His professional career covers biodiversity-related work on projects throughout Africa and its islands (Angola, Botswana, Cameroon, Côte d'Ivoire, Guinea, Lesotho, Liberia, Madagascar, Malawi, Mali, Marion Island, Mozambique, Namibia, South Africa, Uganda, and Zimbabwe). Luke currently co-owns and co-directs the Enviro-Insight consultancy (<http://www.enviro-insight.co.za>) where he fulfills roles as Director, senior ecological specialist, project manager, software developer, and GIS specialist.



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Value of forest remnants for montane amphibians on the livestock grazed Mount Mbam, Cameroon

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Abstract.—Habitat loss and degradation are the primary threats to biodiversity, especially for amphibians. In the Highlands of Cameroon, knowledge on the impacts of different forms of habitat loss, such as livestock management, is restricted to anecdotal reports. This study investigated the impact of forest fragmentation, driven primarily by livestock grazing, on the amphibian assemblage on Mount Mbam, West Region, Cameroon. Stratified, multi-season surveys over two years recorded the abundance and community composition of anuran species. Based on the revised inventory of amphibians the proportion of threatened species on Mount Mbam was calculated at 23.52%. A small population of *Phrynobatrachus steindachneri* was found to occur despite having completely disappeared on other mountains in its distribution range. One species known to the mountain, *Cardioglossa schioetzi*, was not found during the surveys. The remaining forest patches were found to be significant habitat for several species endemic to the mountains of Cameroon-Nigeria. The savanna, likely expanded by livestock grazing, held numerous reed frog species that likely benefit from forest loss, especially in low- to mid-range elevations. The observed relationship between land-use and amphibians on this mountain indicates that the ongoing conversion of forest to pasture threatens remaining montane endemic anuran species, with conservation planning and action now necessary.

Keywords. Africa, endemic, frog, grassland, habitat fragmentation, habitat loss

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Introduction

Vertebrate animal taxa are disappearing worldwide at high rates; especially amphibians, a group which has a high proportion of threatened species (Stuart et al. 2004; Beebee and Griffiths 2005). The five main factors believed to be driving global amphibian declines are the introduction of alien species, habitat alteration, over-exploitation, global change, and infectious diseases (Collins and Storfer 2003). The most severe stressor is physical habitat degradation and destruction (Noss et al. 1997; Stuart et al. 2006). These factors have been found in combination with enigmatic amphibian declines, occurring particularly at localities above 400 m asl in the Americas, Europe, and Australia (Pounds et al. 2006; Vences and Kolher 2008). However, studies on amphibians in African mountains are limited to a handful of sites, although recent findings have shown declines in the Cameroonian mountains that are either enigmatic (possibly associated with chytridiomycosis) or linked to

habitat loss (Hirschfeld et al. 2016; Tchassem et al., in press).

Cameroon is one of the most diverse countries in terms of amphibian species, and it is home to about 4% of the world's known species of frogs (IUCN 2017). The Bamenda Highlands form the northern portion of the Highlands of Cameroon that includes Mount Mbam. Some species known to occur in Mount Mbam are only known from one or a few mountains, including some species classified as Vulnerable or Endangered in the IUCN Red List. This mountain has been studied less extensively than many others, but its study should be a priority given the community level amphibian declines in neighboring mountains (Hirschfeld et al. 2016; Tchassem et al., in press), where the causes remain uncertain as to whether they are habitat driven or the result to other factors such as the chytrid fungus (*Batrochochytrium dendrobatidis* [Bd]). The impacts of environmental change on the Cameroon mountains are still unclear, especially regarding habitat loss, due to limited studies across Sub-Saharan Africa in

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general (Buckley and Jetz 2007).

Deforestation rates are exceptionally high across West and Central Africa (Hansen et al. 2013). In the biodiverse highlands of Cameroon and Nigeria (Bergl et al. 2007), forest loss is driven by a combination of clearance for cultivation, overexploitation of wood fuel, and incursions by livestock (Chapman et al. 2004). The impact of this loss of forest on amphibians has so far been assumed to be the loss of montane endemic species, but the specific factors driving the declines that are associated with forest loss have not been determined. This uncertainty includes the potentially differential impacts of livestock grazing versus cultivation, for example, with potential exacerbation by agrochemicals (Tchassem et al., in press). This scenario is especially applicable to the degradation of natural habitats in the Bamenda Highlands of Cameroon, that include Mounts Oku, Bamboutos, Lefo, and Mbam, which share similar montane endemic species (Cheek et al. 2000; Doherty-Bone and Gvoždík 2017). Montane forest cover has decreased dramatically during the 20th and 21st centuries; with remaining forests becoming highly fragmented (Abbot et al. 2001; Doherty-Bone and Gvoždík 2017). Agrochemical pollution and grazing have not been systematically studied in this area, and since they often occur simultaneously, differentiating the impacts of each stressor is difficult (Tchassem et al., in press).

Mount Mbam has historically been appraised for amphibian diversity, with several montane endemic species identified in the 1970s, such as Perret's Egg Frog (*Leptodatylodon perreti*) and Steindachner's Puddle Frog (*Phrynobatrachus steindachneri*) [Amiet 1971, 1973, 1976]. Despite this work, the mountain has no official protection, and deforestation remains unchecked. Unlike other mountains in the Bamenda Highlands, Mount Mbam does not have considerable cultivation of crops in contrast to its widespread livestock grazing. This study focuses on Mount Mbam to assess the status of the amphibian community and threats from encroachment by livestock grazing. Grazed areas and remnant forests were surveyed, incorporating an elevational gradient, to determine: (1) whether the diversity of amphibians has been altered compared to historical records; and (2) which amphibian species are excluded from the grazed areas compared to the gallery forest.

Materials and Methods

Study area. Mount Mbam (sometimes known as the Mbam Hill Forest), is a calcareous massif located near the town of Fouban in the West Region of Cameroon (Fig. 1, 05°57'N, 10°44'E). It rises from the savanna plains at 1,000 m to a summit of 2,100 m asl. The vegetation of the mountain is transitional between montane savanna grassland mixed with patches of gallery forest (Fotso

et al. 2001; Fig. 2). The forest patches are dominated by *Albizia gummifera*, *Polyscias fulva*, and *Schefflera mannii* (Fotso et al. 2001). Human communities living around and using the mountain include the Bansa'o, Haoussa, and Fulani peoples. Livestock grazing is predominately practiced by the Fulani community, who are semi-nomadic, and concentrated at higher elevations where they live for nine months during the year, from April to December. During January to March (the dry season), these cattle herders move down the mountain to other pastures at lower altitudes.

Study design. To ensure a representative appraisal of diversity, the Mount Mbam amphibian fauna was surveyed over a period of two years, between 2014 and 2016. Field surveys were deployed, with all sample sites visited at least three times. During diurnal and nocturnal visual encounter surveys (VES) with a total duration of 96 surveyor-hours across each land use type were used to quantitatively sample fossorial, arboreal, and water-associated amphibian species (Rödel and Ernst 2004). Nocturnal acoustic encounter surveys were also used to detect animals. Opportunistic sampling was made for all taxa throughout the survey to enable additions to the updated inventory. All sampling sites were characterized with the following environmental data: altitude and coordinates (recorded with a Garmin® GPS extender 90), presence of potential breeding sites, regime of disturbance, and vegetation structure. The vegetation was determined at three strata (canopy cover, shrubs, and understory). Surveys were stratified between gallery forest (the only remnant forest on the mountain) and grassland (primarily grazed by livestock). Surveys involved searching of microhabitats, such as lifting rocks and logs, peeling away bark from trees, moving fallen debris, and inspecting tree stems during daytime (07:00–12:00 h) and night time (19:00–00:00 h) along streams, ponds, and the surrounding vegetation (Crump and Scott 1994; Rödel and Ernst 2004). Amphibians were captured by hand, and then identified and released where they were found. However, a subset of 1–3 individuals of each species, and specimens difficult to identify in the field, were euthanized using an overdose of MS-222 or chlorobutanol solution and preserved in 75% ethanol. These specimens have been deposited in the University of Yaoundé I, Laboratory of Zoology. For species identification, original descriptions and derived literature were used (Perret 1966; Amiet 1977, 1980, 2012; Schiøtz 1999).

Data Analysis. The proportion of threatened species in the total amphibian species inventory for Mount Mbam (both historical and contemporary species observed) was calculated following Böhm et al. (2013). All other analyses were performed with the statistical package R version 3.3.2 (R Core Team 2016). Inventory completeness was assessed using an incidence-based

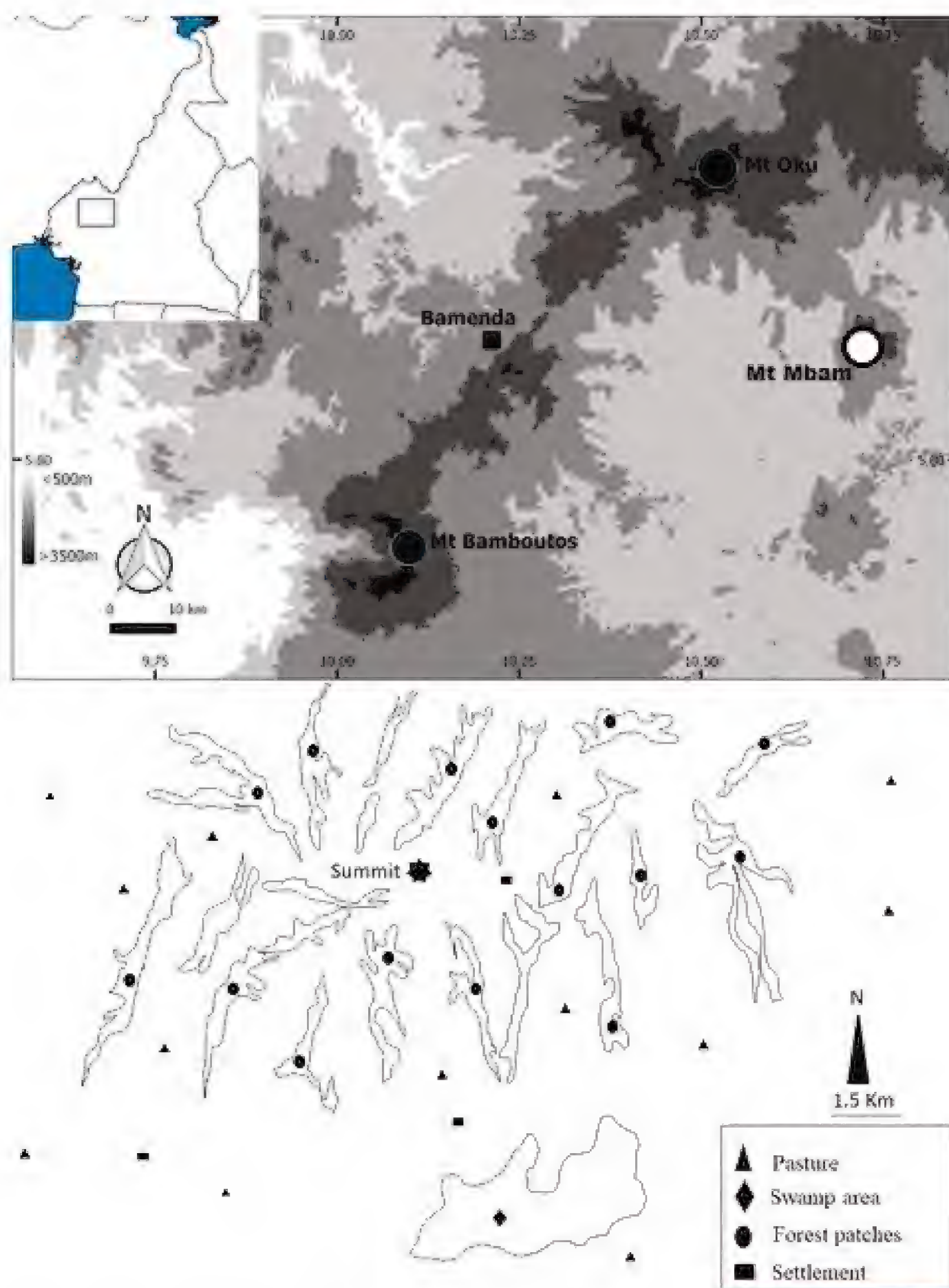


Fig. 1. Maps showing (top) the topography of the Bamenda Highlands, white circle showing Mount Mbam in the West Region of Cameroon; and (bottom) the layout of sample sites on Mount Mbam.

estimator with the package BiodiversityR (Colwell and Coddington 1994). To compare the amphibian community composition between habitat types, PERMANOVA analysis (formula: Adonis, library: vegan) was used to test the significance of the Bray-Curtis dissimilarity based on 999 permutations (Anderson 2001; Riemann et al. 2017). Amphibian abundance data transformed by square root were subjected to ordination analysis using non-metric scale (NMDS) plots of Bray-Curtis dissimilarity (formula: metaMDS, library: vegan) to view the dispersion of similarities. The species contribution to differences between different types of habitats was evaluated by SIMPER analysis (Clarke 1993). The influence of habitat (forest patches, pasture-grassland) on species abundance was evaluated using Generalized Linear Models (GLM, formula: glm, data family:

poisson) [O'Hara and Kotze 2010]. Various parameters such as season, year, and elevation were incorporated into the GLMs to assess potential confounding factors against the response variables (abundance of frogs). GLMs were restricted to species for which a minimum of five individuals were recorded in the surveys. Information criterion analysis was then applied to the GLMs, in which derived Akaike's Information Criteria were used to assess the best performing model based on incorporation of these potentially competing explanatory factors (Mazerolle 2006).

Results

The surveys revealed 17 anuran species of seven genera among 225 individuals (Table 2). Based on these and



Fig. 2. Montane habitats of amphibian species observed in recent surveys of Mount Mbam, West-Region, Cameroon: a) gallery forest during the rainy season; b): gallery forest during the dry season after a bushfire; c) savanna area transformed by overgrazing; and d): effects of bushfire started for pasture on the same site during the dry season.

historical records, the proportion of threatened species on Mount Mbam is estimated at 23.52%. However, the species accumulation curve over the survey periods did show a plateau, indicating the discovery of more species with further searching is unlikely (Fig. 3). In comparison to historical records, one species missing from the contemporary surveys was *Cardioglossa schioetzi* (Table 1). The Puddle Frog, *Phrynobatrachus steindachneri*, was found, but only four sub-adult individuals were observed over the two years. Species new to the historical inventory included the Rocket Frog *Ptychadena mascareniensis* “D” (Zimkus et al. 2016) and the Clawed Frog *Xenopus* cf. *eysoole* (Fig. 4m). The latter species was found as high as 1,600 m asl in stagnant water bodies (including wells) that were heavily frequented by local people and livestock.

Community structure (measured by Bray-Curtis dissimilarity) between the two habitat types was significantly different (PERMANOVA: $p = 0.01$, Fig. 5). Most amphibian species were found in savanna (13 species, 76.47% of individual frogs), with seven species (16% of individuals) found in forest (Table 2). Gallery forests were dominated by species of Arthroleptidae, notably *Astylosternus montanus*, *L. perreti*, and *A.*

rheophilus, which shared the same breeding sites (streams) [Table 3]. *Leptopelis notatus* was more dominant in forests at lower elevations (1,307–1,340 m). The only anurans of Phryobatrachidae on the mountain were *P. steindachneri* restricted to forests at 1,400–1,800 m (Tables 2–3). The only Hyperoliidae found in the forests were a singleton of *Africalus* aff. *fulvovittatus* (at 1,342 m) and two individuals of *Hyperolius igbettensis* (at 1,342–1,684 m), though they likely spilled over from the grassland areas.

Within derived savanna (i.e., savanna created by cattle grazing and fire) Hyperoliidae (43.55% of all individual frogs) was the most dominant group, represented primarily below 1,500 m by *Hyperolius concolor*, *H. balfouri*, *H. tuberculatus*, *H. igbettensis*, *H. nitidulus*, *H. cinnamomeoventris*, and *A. aff. fulvovittatus* (Tables 2–3). This family was followed by Pipidae (25.8% of individuals, represented by *X. cf. eysoole* only), Arthroleptidae (7% for *L. notatus*, *L. nordequatorialis*, as well as a minority of five individual *A. montanus*), Ptychadenidae (5.8%, represented by *P. mascareniensis* “D” only), Bufonidae (2.2% for *Sclerophrys maculata* only), and Dicroglossidae (2.2% for *Hoplobatrachus occipitalis* only). Four species occurred in both habitats: *A. aff. fulvovittatus*, *A. montanus*,

Table 1. An updated amphibian species inventory for Mount Mbam, Cameroon.

Anuran taxa	Species authority	Global IUCN status	Endemicity	References
Arthroleptidae				
<i>Astylosternus montanus</i>	Amiet, 1978	LC	CNH	Amiet 1978; present study
<i>Astylosternus rheophilus</i>	Amiet, 1977	VU	CNH	Amiet 1977; Hirschfeld et al. 2016; present study
<i>Cardioglossa schioetzi</i>	Amiet, 1982	VU	CNH	Amiet 1982; Schiøtz 2004
<i>Leptodactylodon perreti</i>	Amiet, 1971	EN	BamH	Amiet 1980; present study
<i>Leptopelis nordequatorialis</i>	Perret, 1966	LC	CNH	Perret 1966; Amiet 1971, 1974, 1980; Gartshore 1986; present study
<i>Leptopelis notatus</i>	(Peters, 1875)	LC	S-Sa	Boulenger 1906; Nieden 1909; Goin 1961; present study
Bufonidae				
<i>Sclerophrys maculata</i>	(Hallowell, 1854)	LC	PanAfr	Hirschfeld et al. 2016; present study
<i>Hoplobatrachus occipitalis</i>	Günther, 1858	LC	S-Sa	present study
Hyperolidae				
<i>Afrixalus</i> aff. <i>fulvovittatus</i>	Pickersgill, 2007	LC	S-Sa	Perret 1976; present study
<i>Hyperolius balfouri</i>	Werner, 1907	LC	S-Sa	Werner 1908; Scortecci 1943; Monard, 1951; present study
<i>Hyperolius</i> cf. <i>cinnamomeoventris</i>	Bocage, 1866	LC	S-Sa	Inger 1968; Largen and Dowsett-Lemaire 1991; Schiøtz 1999; present study
<i>Hyperolius igbettensis</i>	Mertens, 1940	LC	S-Sa	Schiøtz 1963; present study
<i>Hyperolius nitidulus</i>	Peters, 1875	LC	S-Sa	Perret 1966; present study
<i>Hyperolius tuberculatus</i>	Mocquard, 1897	LC	S-Sa	present study
Pipidae				
<i>Xenopus</i> cf. <i>eysoole</i>	Evans et al. 2015			present study
Phrynobatrachidae				
<i>Phrynobatrachus steindachneri</i>	Neiden, 1910	VU	CNH	Mertens 1968; Hirschfeld et al. 2016
Ptychadenidae				
<i>Ptychadena mascareniensis</i> “D”	Zimkus et al. 2017	LC	S-Sa	present study

Endemicity codes for species limited to: S-Sa - sub-Saharan Africa; C.W.A+Ng - Central and West African countries and Nigeria; BamH – just the Bamenda Highlands of Cameroon that includes Mount Mbam; CNH: just the Bamenda Highlands of Cameroon and Nigeria.

H. igbettensis, and *L. notatus*.

Species represented by at least five individuals, regardless of habitat type, varied with the strength of the models in relation to habitat type. Species usually associated with forest had better fitted models with habitat (Δ AIC 0–7) and significant *p*-values, notably *Astylosternus* sp. and *L. perreti*, as well as some savanna species such as *P. mascariensis* “D,” *H. nititdulus*, and *H. tuberculatus* (Table 4). Species associated with savanna (*Afrixalus*, some *Hyperolius* sp., and *S. maculata*) and *L. notatus*, however, did not show statistically significant or well fitted models with habitat as a fixed variable (Table 4). Habitat**elevation* and season**elevation* both provided the best fitted models (the lowest AIC) for *A. rheophilus*, with a Δ AIC of seven from the inclusion of habitat alone or from the inclusion of habitat**elevation***season*. Habitat is a major contributor for the best fitted models for the species *A. montanus* (habitat**year*), *L. notatus* (habitat**elevation*), and *P. mascareniensis* “D” (habitat**season*) [Table 4]. Year of survey did have an influence over the fit of the models, with the exceptions of *H. cinnamomeoventris*, *P. mascareniensis*, and *S.*

maculata, manifest by all three having fewer records for the year 2016. For the remaining species, the lack of an influence of year indicates population stability in this two-year time period on the mountain.

Discussion

This study quantitatively assessed the status and habitat use of amphibians on Mount Mbam, Cameroon, in relation to land use. The species inventory of the amphibians of the mountain was updated, revealing little additional diversity recorded for the Mbam massif so far, beyond more lowland-adapted species. The stratified survey enabled better understanding of the habitat requirements of numerous amphibian species, especially montane species with restricted ranges. Species composition varied considerably between montane savanna and forest habitats. With a higher elevation, the forest was generally found to have fewer species, but with considerably more numerous montane endemic species. There were clear instances of spillover from one habitat to another, typified by the occurrences of a minority of species in one habitat compared to the habitat in which they are numerically

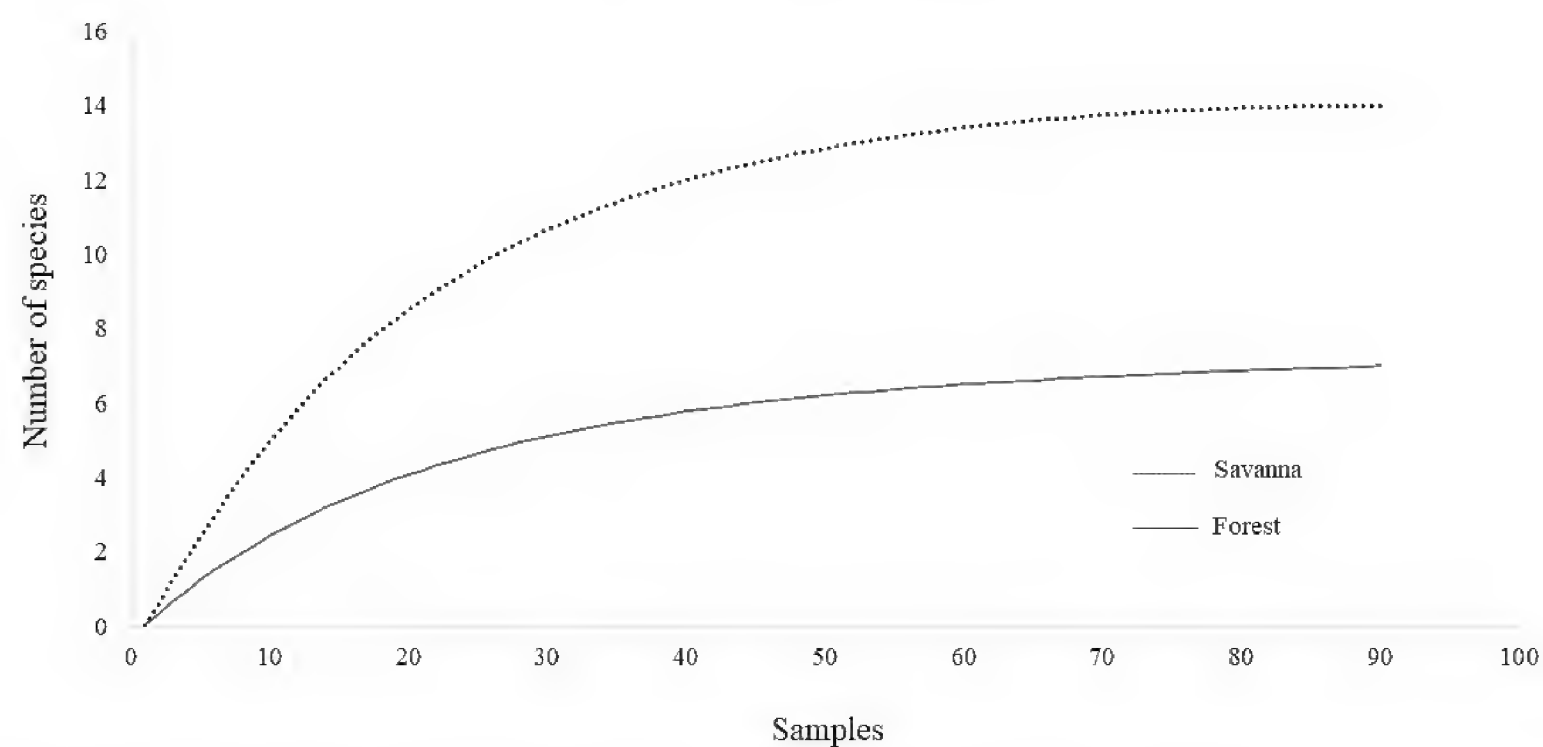


Fig. 3. Species accumulation curves of Mount Mbam by land use based on contemporary records.

dominant.

Compared to nearby mountains such as Oku or Bamboutos, Mount Mbam has a more diverse community of hyperoliids, likely due to a greater area of the mountain consisting of lower elevations. This is in contrast to mountains such as Bamboutos, where agrochemicals are widely used. It is notable that the genera *Arthroleptis* and *Kassina* were not observed in either historical or contemporary surveys, and the explanation for their absence is unclear. Montane endemic species known to Mounts Lefo, Oku, and Bamboutos, including *Cardioglossa oreas*, *C. pulchra*, and *Astylosternus ranoides*, were not found on Mbam. This is despite other species such as *Leptodactylodon perreti* and *A. rheophilus* occurring on this mountain, and the fact that these absent species do have elevational ranges corresponding to that of Mt. Mbam. This suggests the possibilities that they are either: (i) locally extinct through human land use practices or climate change that pre-date the first surveys of the mountain in the 1960s, or (ii) that the mountain is too small and low in elevation for viable populations of the higher montane endemic frog species to persist.

Despite its disappearances on Mount Oku in the North West Region and Mount Bamboutos in the West Region (Doherty-Bone and Gvoždík 2017; TchasseM et al., in press), *P. steindachneri* was observed during the recent field surveys. This taxon is part of a species complex that could possibly also include *P. jimzimkusi* (Zimkus and Gvoždík 2013). *Cardioglossa schioetzi* was not observed in this study, and it was also not found on Mount Oku in recent years, as with certain other species of *Cardioglossa*, *Werneria*, and *Phrynobatrachus* (Hirschfeld et al. 2016; Doherty-Bone and Gvoždík 2017; TchasseM et al., in press). The causes of these disappearances remain unknown, but the declines on Mount Manengouba and Mount Oku have coincided with an increase in the prevalence of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, *Bd*), indicating that disease could be a factor (Hirschfeld et al. 2016).

The role of climate change in these declines also remains unclear and represents a research gap that requires urgent attention (Doherty-Bone and Gvoždík 2017; TchasseM et al., in press). On Mount Mbam, further research should include investigating the role of fire, which could be a factor, not just from its use by livestock herders but

Table 2. Summary of amphibian species (total per habitat type across all survey techniques) encountered during the present study (2014–2016).

Species	Pasture-grassland	Gallery forest
<i>Afrivalus</i> aff. <i>fulvovittatus</i>	15	1
<i>Astylosternus montanus</i>	5	14
<i>Astylosternus rheophilus</i>	0	5
<i>Hoplobatrachus occipitalis</i>	5	0
<i>Hyperolius balfouri</i>	21	0
<i>Hyperolius tuberculatus</i>	15	0
<i>Hyperolius concolor</i>	22	0
<i>Hyperolius igbettensis</i>	7	2
<i>Hyperolius nitidulus</i>	8	0
<i>Hyperolius cinnamomeoventris</i>	7	0
<i>Leptodactylodon perreti</i>	5	0
<i>Leptopelis nordequatorialis</i>	2	0
<i>Leptopelis notatus</i>	6	5
<i>Phrynobatrachus steindachneri</i>	0	4
<i>Sclerophrys maculata</i>	0	5
<i>Ptychadena mascareniensis</i> “D”	13	0
<i>Xenopus</i> cf. <i>eysoole</i>	58	0
Total	189	36
Species richness	14	7
Species evenness	0.86	0.80
Shannon’s <i>D</i>	3.27	2.44
Mean number of specimens per sampling event	23.63	4.50



Fig. 4. Montane endemic amphibian species observed in recent surveys of Mount Mbam, West-Region, Cameroon. **a)** *Astylosternus rheophilus*, **b)** *Astylosternus montanus*, **c)** *Afrixalus* aff. *fulvovittatus*, **d)** *Hyperolius balfouri*, **e)** *Hyperolius igbettensis*, **f)** *Hyperolius nitidulus*, **g)** *Hyperolius concolor*, **h)** *Hyperolius cinnamomeoventris*, **i)** *Hyperolius tuberculatus*, **j)** *Leptopelis nordequatorialis*, **k)** *Leptopelis boulengeri*, **l)** *Phrynobatrachus steindachneri*, **m)** *Xenopus* cf. *eysoole*, **n)** *Hoplobatrachus occipitalis*, and **o)** *Sclerophrys maculata*.

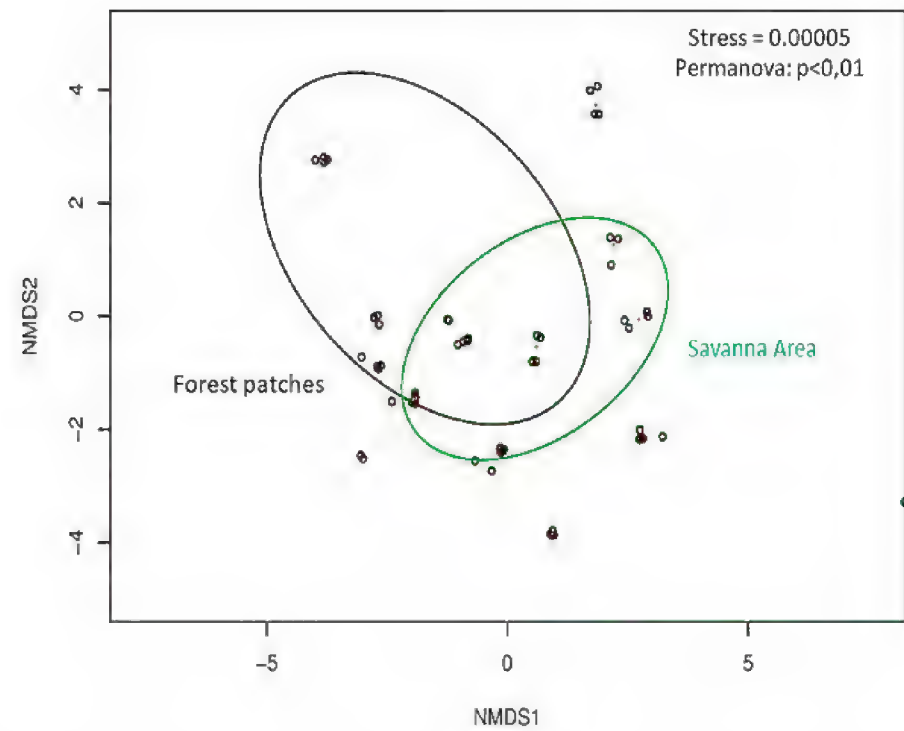


Fig. 5. Non-metric dimensional scaling plot of amphibian community structure divided by land use type on Mount Mbam based on visual encounter surveys with equal effort for each land use. The PERMANOVA *p*-value is shown in the top right corner.

also its influence by climate change. Some species were possibly overlooked during this study, possibly due to variance in the detectability of rare and/or cryptic species (Mackenzie et al. 2005; Megson et al. 2009); thus, further efforts to continue this study would benefit from complementary survey methods such as pitfall traps and continuous audio recordings. However, it is also possible that the species pool for Mount Mbam has simply been degraded historically.

The presence of livestock grazing on the slopes of Mount Mbam has likely modified the landscape structure of habitats to a great extent, such as through loss of

forest from grazing, fires, and trampling by livestock (Carte and John 2002). The remaining forest on Mount Mbam is now reduced to patches along streams and close to the summit where access is difficult for livestock. The historical loss of forest is likely to have negatively affected populations of forest-dependent species, while potentially benefitting tolerant, savanna-adapted species. While the forests hosted fewer species than savanna, those species are montane endemics of conservation concern, while the greater species richness of the savanna consists of species with broader, lowland ranges. Most of the amphibian species were significantly influenced by elevation, habitat, and season. This indicates that despite more savanna becoming available, the colonization by lowland species may remain limited.

Planning and implementing effective strategies to control habitat disturbance and encourage recovery on this mountain could be required to stop further loss of montane endemic species in the long term. Future studies should investigate the precise impacts of bushfire, including influences on nutrient cycling, water quality, predation risk to anurans, and reproductive success. The conservation needs of Mount Mbam are similar to those of Mount Bamboutos, but are driven more by livestock than the cultivation of crops. As with Mount Bamboutos, Mount Mbam has neither official protection nor conservation action, and the exploitation of its resources is unregulated. Several measures could be implemented to reduce the rate of forest loss on Mount Mbam. Steps such as raising environmental awareness, conducting educational seminars, and preparing educational materials for the locals, would certainly have a positive

Table 3. Similarity percentage (SIMPER) analysis showing importance of dissimilarity for various amphibian species for habitat type based on visual encounter surveys of Mount Mbam.

Taxon	Mean number of individuals per survey			
	Forest	Pasture-grassland	Contribution to dissimilarity	Cumulative contribution
<i>Xenopus</i> cf. <i>eysoole</i>	0 ± 0	0.64 ± 1.24	0.17	0.17
<i>Astylosternus montanus</i>	0.47 ± 0.86	0.06 ± 0.23	0.14	0.32
<i>Leptopelis notatus</i>	0.17 ± 0.42	0.07 ± 0.39	0.08	0.39
<i>Hyperolius balfouri</i>	0 ± 0	0.23 ± 0.75	0.07	0.46
<i>Hyperolius concolor</i>	0 ± 0	0.24 ± 0.96	0.06	0.58
<i>Africalus</i> aff. <i>fulvovittatus</i>	0.03 ± 0.18	0.17 ± 0.60	0.06	0.52
<i>Astylosternus rheophilus</i>	0.17 ± 0.46	0 ± 0	0.05	0.69
<i>Hyperolius igbettensis</i>	0.07 ± 0.25	0.08 ± 0.37	0.05	0.79
<i>Hyperolius tuberculatus</i>	0 ± 0	0.17 ± 0.64	0.05	0.89
<i>Phrynobatrachus steindachneri</i>	0.13 ± 0.35	0 ± 0	0.05	0.84
<i>Ptychadena mascareniensis</i>	0 ± 0	0.14 ± 0.49	0.05	0.63
<i>Hyperolius cinnamomeoventris</i>	0 ± 0	0.08 ± 0.37	0.03	0.95
<i>Hyperolius nitidulus</i>	0 ± 0	0.09 ± 0.41	0.03	0.92
<i>Sclerophrys maculata</i>	0 ± 0	0.06 ± 0.23	0.03	0.97
<i>Hoplobatrachus occipitalis</i>	0 ± 0	0.06 ± 0.38	0.02	0.99
<i>Leptopelis nordequatorialis</i>	0 ± 0	0.02 ± 0.15	0.01	1

Amphibians of Mount Mbam, Cameroon

Table 4. Generalized linear models comparing parameters which influence the abundance of amphibian species on Mount Mbam.

Species	Model parameters	df	Residual deviance	p-value	AIC	ΔAIC
<i>Afrixalus</i> aff. <i>fulvovittatus</i>	Habitat*Elevation*Season	112	41.48	0.99	78	2
	Habitat*Elevation	116	63.52	< 0.01	92	26
	Habitat*Season	116	62.95	<0.001	91	25
	Season*Elevation	116	47.88	<0.001	76	0
	Habitat*Year	116	77.78	0.01	106	30
	Habitat	118	84.83	0.05	109	33
	Elevation	118	74.05	<0.001	98	12
	Season	118	81.87	<0.01	106	30
	Year	112	88.35	0.53	112	36
<i>Astylosternus montanus</i>	Habitat*Elevation*Season	112	51.96	0.99	100	7
	Habitat*Elevation	116	59.92	0.06	100	7
	Habitat*Season	116	56.69	0.99	97	4
	Season*Elevation	116	64.91	0.99	105	8
	Habitat*Year	116	53.47	0.90	93	0
	Habitat	118	64.11	<0.001	100	7
	Elevation	118	75.79	<0.01	112	19
	Season	118	68.49	<0.001	104	11
	Year	118	81.52	0.12	117	24
<i>Astylosternus rheophilus</i>	Habitat*Elevation*Season	112	9.76	0.99	99	7
	Habitat*Elevation	116	9.76	0.99	26	0
	Habitat*Season	116	20.00	0.99	37	11
	Season*Elevation	116	9.92	1	26	0
	Habitat*Year	116	20.51	0.99	37	11
	Habitat	118	20.69	<0.001	33	7
	Elevation	118	9.93	<0.001	58	32
	Season	118	30.50	<0.001	43	17
	Year	118	31.11	0.51	45	19
<i>Hoplobatrachus occipitalis</i>	Habitat*Elevation*Season	112	34.40	1.00	56	13
	Habitat*Elevation	116	35.47	1.00	49	6
	Habitat*Season	116	38.26	0.99	52	9
	Season*Elevation	116	34.65	0.32	48	5
	Habitat*Year	116	29.11	1.00	43	0
	Habitat	118	38.268	0.09	48	5
	Elevation	118	35.64	0.02	45	2
	Season	118	41.05	0.75	50	7
	Year	118	204.44	0.21	269.90	221
<i>Hyperolius balfouri</i>	Habitat*Elevation*Season	112	68.50	0.99	108	29
	Habitat*Elevation	116	90.26	1.00	123	21
	Habitat*Season	116	76.22	0.99	108	6
	Season*Elevation	116	69.92	0.99	102	0
	Habitat*Year	116	99.10	0.99	131	29
	Habitat	118	99.26	<0.001	128	26
	Elevation	118	91.12	<0.001	119	30

Table 4 (Cont.). Generalized linear models comparing parameters which influence the abundance of amphibian species on Mount Mbam.

Species	Model parameters	df	Residual deviance	p-value	AIC	ΔAIC
<i>Hyperolius cinnamomeoventris</i>	Season	118	94.31	<0.001	123	21
	Year	118	111.34	0.94	139	36
	Habitat*Elevation*Season	112	39.44	1.00	65	19
	Habitat*Elevation	116	42.10	0.99	60	14
	Habitat*Season	116	44.07	1.00	62	16
	Season*Elevation	116	40.14	0.13	58	12
	Habitat*Year	116	31.24	1.00	49	3
	Habitat	118	44.07	0.04	58	12
	Elevation	118	42.49	0.02	56	10
<i>Hyperolius concolor</i>	Season	118	47.83	0.60	62	16
	Year	118	32.36	<0.001	46	0
	Habitat*Elevation*Season	112	82.19	0.99	119	0
	Habitat*Elevation	116	103.88	0.99	132	13
	Habitat*Season	116	108.83	0.99	137	19
	Season*Elevation	116	100.95	<0.001	129	10
	Habitat*Year	116	116.84	0.99	145	26
	Habitat	118	117.32	<0.001	142	23
	Elevation	118	129.98	0.97	154	35
<i>Hyperolius igbettensis</i>	Season	118	125.50	0.03	150	31
	Year	118	129.81	0.68	154	35
	Habitat*Elevation*Season	112	044.07	0.19	74	2
	Habitat*Elevation	116	53.05	0.36	75	3
	Habitat*Season	116	52.12	0.13	74	2
	Season*Elevation	116	53.54	0.46	75	3
	Habitat*Year	116	54.83	0.81	76	4
	Habitat	118	54.91	0.84	73	1
	Elevation	118	54.30	0.42	72	0
<i>Hyperolius nitidulus</i>	Season	118	54.46	0.49	72	0
	Year	118	54.93	0.91	73	1
	Habitat*Elevation*Season	112	43.35	1.00	71	7
	Habitat*Elevation	116	48.10	0.99	68	4
	Habitat*Season	116	44.71	1.00	64	0
	Season*Elevation	116	46.39	0.30	66	2
	Habitat*Year	116	46.23	0.99	65	1
	Habitat	118	48.10	0.03	64	0
	Elevation	118	50.29	0.12	66	2
<i>Hyperolius tuberculatus</i>	Season	118	50.85	0.17	66	2
	Year	118	49.48	0.07	65	1
	Habitat*Elevation*Season	112	73.36	0.99	106	7
	Habitat*Elevation	116	79.67	0.99	104	5
	Habitat*Season	116	75.91	0.99	100	1
	Season*Elevation	116	74.91	0.33	99	0
	Habitat*Year	116	81.60	0.99	106	7

Amphibians of Mount Mbam, Cameroon

Table 4 (Cont.). Generalized linear models comparing parameters which influence the abundance of amphibian species on Mount Mbam.

Species	Model parameters	df	Residual deviance	p-value	AIC	ΔAIC
<i>Leptodactylodon perreti</i>	Habitat	118	81.85	<0. 01	103	4
	Elevation	118	80.99	<0.01	101	2
	Season	118	87.32	<0. 01	108	9
	Year	118	90.41	0.79	111	12
	Habitat*Elevation*Season	112	14.52	0.99	39	11
	Habitat*Elevation	116	14.54	0.99	31	3
	Habitat*Season	116	20.00	1.00	37	9
	Season*Elevation	116	14.99	0.99	32	4
	Habitat*Year	116	20.51	0.99	37	9
<i>Leptopelis notatus</i>	Habitat	118	20.69	<0.001	33	5
	Elevation	118	15.07	<0.001	28	0
	Season	118	30.49	0.04	43	15
	Year	118	34.12	0.51	46	18
	Habitat*Elevation*Season	112	31.79	0.99	65	14
	Habitat*Elevation	116	43.04	<0.01	69	0
	Habitat*Season	116	48.74	0.01	74	5
	Season*Elevation	116	48.84	<0.01	74	5
	Habitat*Year	116	58.95	0.85	84	14
<i>Ptychadena mascareniensis</i> “D”	Habitat	118	59.78	0.14	81	12
	Elevation	118	61.16	0.38	83	12
	Season	118	59.86	0.15	81	12
	Year	118	60.98	0.33	82	13
	Habitat*Elevation*Season	112	52.10	1.00	88	4
	Habitat*Elevation	116	61.89	0.99	90	6
	Habitat*Season	116	55.95	0.99	84	0
	Season*Elevation	116	58.16	0.28	86	2
	Habitat*Year	116	57.98	0.99	86	2
<i>Sclerophrys maculata</i>	Habitat	118	62.44	<0.01	87	3
	Elevation	118	67.71	0.14	90	6
	Season	118	59.56	<0.01	84	0
	Year	118	156	<0.001	90	6
	Habitat*Elevation*Season	112	27.81	1.00	54	20
	Habitat*Elevation	116	28.90	1.00	57	23
	Habitat*Season	116	28.27	0.99	46	12
	Season*Elevation	116	28.49	0.33	46	12
	Habitat*Year	116	19.74	0.99	38	4
<i>Xenopus</i> cf. <i>eysoole</i>	Habitat	118	28.90	0.09	43	9
	Elevation	118	30.34	0.23	44	10
	Season	118	30.30	0.22	44	10
	Year	118	20.54	<0.001	34	0
	Habitat*Elevation*Season*Year	-1	0	1.00	148	103
	Habitat*Elevation*Season	112	151.00	1.00	228	183
	Habitat*Elevation	116	172.00	0.99	241	196

Table 4 (Cont.). Generalized linear models comparing parameters which influence the abundance of amphibian species on Mount Mbam.

Species	Model parameters	df	Residual deviance	p-value	AIC	ΔAIC
	Habitat*Season	116	153.46	1.00	223	178
	Season*Elevation	116	160.98	0.008	230	185
	Habitat*Year	116	172.40	1.00	242	197
	Habitat	118	172.65	<0.001	238	193
	Elevation	118	192.65	<0.001	265	220
	Season	118	172.32	<0.001	238	193
	Year	118	204.44	<0.001	45	0

effect on changing negative attitudes. The creation of a protected area for these habitats, with strong involvement of the local people, would be a plausible strategy. The proportion of endangered species of the Mount Mbam is very low, but this does not in any way diminish its importance for the conservation of the endemic amphibian species, whereas the differences observed at low and high altitudes with regard to species composition and habitat type should make this site a national or sub-regional conservation priority.

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Literature Cited

Abbot JIO, Thomas DHL, Gardner AA, Neba SE, Mbony WK. 2001. Understanding the links between conservation and development in the Bamenda Highlands, Cameroon. *World Development* 29: 1,115–1,136.

Amiet J-L. 1971. Les batraciens orophiles du Cameroun. *Annales de la Faculté des Sciences du Cameroun* 5: 83–102.

Amiet J-L. 1973. Notes faunistiques, éthologiques et écologiques sur quelques Amphibiens Anoures du Cameroun (2e série). *Annales de la Faculté des Sciences du Cameroun* 13: 135–161.

Amiet J-L. 1976. Ecologie et distribution des Amphibiens Anoures de la région de Nkongsamba (Cameroun). *Annales de la Faculté des Sciences du Cameroun* 20: 33–107.

Amiet J-L. 1977. Les *Astylosternus* du Cameroun (Am-

phibia, Anura, Astylosternidae). *Annales de la Faculté des Sciences du Cameroun* 23–24: 99–227.

Amiet J-L. 1980. Revision du genre *Leptodactylodon* Andersson (Amphibia, Anura, Astylosternidae). *Annales de la Faculté des Sciences du Cameroun* 27: 69–224.

Amiet J-L. 2012. *Les Rainettes du Cameroun (Amphibiens Anoures)*. Edition JL Amiet, Nyons and La Nef de Livres, Saint-Nazaire, France. 591 p.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26: 32–46.

Bergl RA, Oates JF, Fotso R. 2007. Distribution and protected area coverage of endemic taxa in West Africa’s Biafran forests and highlands. *Biological Conservation* 134: 195–208.

Böhm M, Collen B, Baillie JEM, Bowles P, Chanson J, Cox N, Hammerson G, Hoffmann M, Livingstone SR, Ram M, et al. 2013. The conservation status of the world’s reptiles. *Biological Conservation* 157: 372–385.

Buckley LB, Jetz W. 2007. Environmental and historical constraints on global patterns of amphibian richness. *Proceedings of the Royal Society B* 274: 1,167–1,173.

Chapman HM, Olson SM, Trumm D. 2004. An assessment of changes in the montane forests of Taraba State, Nigeria, over the past 30 years. *Oryx* 38: 282–290.

Cheek M, Onana JM, Pollard BJ. 2000. *The Plants of Mount Oku and the Ijim Ridge, Cameroon. A Conservation Check-list*. Royal Botanic Gardens Kew, London, United Kingdom. 215 p.

Clarke KR. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18: 117–143.

Collins JP, Storfer A. 2003. Global amphibian declines: sorting the hypotheses. *Diversity and Distributions* 9: 89–98.

Colwell RK, Coddington JA. 1994. Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions of the Royal Society of London. Series B* 345: 101–118.

Crump ML, Scott NJ. 1994. Visual encounter surveys. Pp. 84–92 In: *Measuring and Monitoring Biological*

- Diversity. Standard Methods for Amphibians*. Editors, Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS. Smithsonian Institution Press, Washington, DC, USA. 364 p.
- Doherty-Bone TM, Gvoždík V. 2017. The amphibians of Mount Oku, Cameroon: an updated species inventory and conservation review. *ZooKeys* 643: 109–139.
- Fotso R, Dowsett-Lemaire F, Dowsett RJ, Cameroon Ornithological Club, Scholt EP, Languy M, Bowden C. 2001. Cameroon. Pp. 133–159 In: *Important Bird Areas in Africa and Associated Islands: Priority Sites for Conservation*. Editors, Fishpool LDC, Evans MI. Birdlife Conservation Series Number 11. Priscas Publica, Newbury and Cambridge, United Kingdom. 1,144 p.
- Hansen MC, Potapov PV, Moore R, Hancher M, Turubanova SA, Tyukavina A, Thau D, Stehman SV, Goe SJ. 2013. High-resolution global maps of 21st-century forest cover change. *Science* 342: 850–853.
- Hirschfeld M, Blackburn DC, Doherty-Bone TM, Gonwouo LN, Ghose S, Rödel M-O. 2016. Dramatic declines of montane frogs in a central African biodiversity hotspot. *PLOS One* 11(5): e0155129.
- Mackenzie DI, Nichols JD, Sutton N, Kawanishi K, Bailey LL. 2005. Improving inferences in population studies of rare species that are detected imperfectly. *Ecology* 86: 1,101–1,113.
- Mazerolle MJ. 2006. Improving data analysis in herpetology: using Akaike's Information Criterion (AIC) to assess the strength of biological hypotheses. *Amphibia-Reptilia* 27: 169–180.
- Megson S, Mitchell P, Köhler J, Marsh C, Franzen M, Glaw F, D'Cruze N. 2009. A comprehensive survey of amphibians and reptiles in the extreme north of Madagascar. *Herpetological Notes* 2: 31–44.
- Noss RF, O'Connell MA, Murphy DD. 1997. *The Science of Conservation Planning*. Island Press, Washington, DC, USA. 263 p.
- O'Hara RB, Kotze DJ. 2010. Do not log-transform count data. *Methods in Ecology and Evolution* 1: 118–122.
- Perret J-L. 1966. Les amphibiens du Cameroun. *Zoologische Jahrbücher für Systematik* 8: 289–464.
- Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Fogden MPL, Foster PN, La Marca E, Masters K, Merino-Viteri A, Puschendorf R, et al. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439: 161–167.
- R Core Team. 2016. *R: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available: <http://www.R-project.org/> [Accessed: 30 July 2017].
- Riemann JC, Ndriantsoa SH, Rödel M-O, Glos J. 2017. Functional diversity in a fragmented landscape—Habitat alterations affect functional trait composition of frog assemblages in Madagascar. *Global Ecology and Conservation* 10: 173–183.
- Schiøtz A. 1999. *Treefrogs of Africa*. Editon Chimaira, Frankfurt am Main, Germany. 350 p.
- Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Walter RW. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1,783–1,786.
- Tchasse AF, Doherty-Bone TM, Kamani MM, Tapondjou WP, Tamesse JL, Gonwouo NL 2019. What is driving declines of montane endemic amphibians? New insights from Mount Bamboutos, Cameroon. *Oryx*, in press.
- Todd BD, Rothermel BB. 2006. Assessing quality of clearcut habitats for amphibians: Effects on abundances versus vital rates in the Southern Toad (*Bufo terrestris*). *Biological Conservation* 133: 178–185.
- Vences M, Köhler J. 2008. Global diversity of amphibians (Amphibia) in freshwater. *Hydrobiologia* 595: 569–580.
- Zimkus BM, Gvoždík V. 2013. Sky islands of the Cameroon Volcanic Line: a diversification hot spot for puddle frogs (Phrynobatrachidae: *Phrynobatrachus*). *Zoologica Scripta* 42: 591–611.



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Another Angolan Namib endemic species: a new *Nucras* Gray, 1838 (Squamata: Lacertidae) from south-western Angola

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Abstract.—A new endemic Sandveld Lizard, genus *Nucras*, is described from south-western Angola. Morphologically it resembles members of the *Nucras tessellata* group, but it is genetically separated and is sister to the larger *tessellata* + *lalandii* group. Although the genus is generally very conservative morphologically, the new species differs from other congeners in a combination of scalation, overall dorsal color pattern, and geographic separation. The new species is known from fewer than 12 specimens collected over a period spanning 120 years from arid south-western Angola. This brings the total number of species in the genus to 12 and adds another species to the growing list of endemic species of the Namib region of Angola. This new finding further reinforces the idea that this Kaokoveld Desert region is a key biodiversity area worthy of conservation and long-term protection.

Keywords. Sandveld Lizard, taxonomy, Africa, endemism, Kaokoveld, biodiversity hotspot

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Introduction

The recorded reptile diversity in Angola (278 species, Marques et al. 2018; Branch et al. 2019) is significantly lower than that of South Africa (407 species, Tolley et al. 2019), a nearby country of comparable size and habitat diversity. This incongruity has been attributed to the lack of recent faunal surveys and/or taxonomic revision of groups in the country (Marques et al. 2018; Branch et al. 2019). That this gap simply represents under-sampling of the Angolan herpetofauna is evidenced by the recent discovery of numerous new species, including lacertids of genus *Pedioplanis* (Conradie et al. 2012), girdled lizards of genus *Cordylus* (Stanley et al. 2016; Marques et al. 2019b), and a new skink of genus *Trachylepis* (Marques et al. 2019a), as well as several candidate new species of lacertids (Branch and Tolley 2017), and geckos (Branch et al. 2017).

At present, the family Lacertidae is represented in Angola by 13 species in six genera; *Heliobolus* (one species), *Holaspis* (one), *Ichnotropis* (three), *Meroles* (three), *Nucras* (two), and *Pedioplanis* (three; see Marques et al. 2018; Branch et al. 2019). The lacertid generic diversity is comparable to that of other herpetologically rich areas in sub-Saharan Africa, e.g., eight genera in Tanzania, Kenya, and South Africa, and five in Namibia (Branch 1998; Spawls et al. 2018). However, the lacertid species diversity in Angola (13 species) is notably lower: Kenya (15), Tanzania (16), Namibia (25), and South Africa (28) [Branch 1998; Spawls et al. 2018; Branch et al. 2019; Bauer et al. 2019].

The taxonomy of the lacertid genus *Nucras* Gray, 1838 is complicated by the relatively secretive habits and conservative morphology of known species, and this has confounded early attempts to resolve species

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boundaries and geographical distributions within the genus. Currently, *Nucras* comprises eleven species that are mainly restricted to southern Africa, with a northern outlier (*Nucras boulengeri* Neumann, 1900) occurring in East Africa, although there is a single, isolated record from Isoka, northern Zambia (Haagner et al. 2000; Spawls et al. 2018).

Taxonomy of the Western (or Striped) Sandveld Lizard, *Nucras tessellata*, has proven to be particularly problematic, as have the species boundaries within the *N. tessellata* species complex. Broadley (1972) recognized four subspecies (*Nucras taeniolata taeniolata*, *Nucras taeniolata ornata*, *Nucras tessellata tessellata*, and *Nucras tessellata livida*), as well as a number of taxonomically unresolved non-specific forms, i.e., *Nucras tessellata tessellata* var. “T,” *Nucras taeniolata ornata* var. *holubi*, and *Nucras tessellata tessellata* var. *elegans*. Broadley (1972) examined the morphology of over 800 specimens and concluded that the dorsal color pattern and the number of subdigital lamellae under the 4th toe are reliable taxonomic characters to differentiate species within the *N. tessellata* complex. In recent years, several subspecies and varieties were elevated to full species, e.g., *Nucras taeniolata*, *N. holubi*, *N. ornata* (Jacobsen 1989), and *N. livida* (Branch and Bauer 1995). A number of historically problematic Angolan specimens were considered to form part of the *Nucras tessellata* (Smith, 1838) complex, best representing *Nucras tessellata tessellata* var. “T.” However, Broadley (1972) deferred making a decision on their taxonomic status pending the collection of additional material. The only other Angolan member of the genus, *Nucras scalaris* Laurent, 1964, was described on the basis of material from northern Angola and is not currently regarded to be included in the *N. tessellata* complex.

Bocage (1895) was the first to record *N. tessellata* from Angola, but noted only that (translated from the original French): “Mr. Anchieta met this species at two different locations, Maconjo and Caconda, from where he sent us a few individuals. All of these individuals belong to the variety *taeniolata*, separated from the typical form not only by its coloration, with a back striped longitudinally in white and blackish-brown, but is also slimmer.” He provided no further details of the specimens, leaving out information on scalation and size. Fortunately, the late Donald G. Broadley visited the Museu Bocage Lisboa, Portugal (currently Museu Nacional de História Natural e da Ciência) in 1968, before the disastrous fire of 1978 destroyed its collections. Broadley was only able to locate the three specimens from Maconjo listed by Bocage (1895). Boulenger (1910) first assigned specimens from Moçâmedes (=Namibe) district to *Nucras tessellata* var. *taeniolata*. In subsequent years, he referred the same material as part of *Nucras intertexta* var. *holubi* under a different color variation A and called this the most ‘primitive form’ (Boulenger 1917, 1921). Monard (1937)

recorded three additional juvenile specimens from Kapelongo (= Capelongo) and reported that they exhibit typical coloration of *taeniolata*, and thus assigned his material to the *N. tessellata* complex. The most detailed description to follow was a specimen collected from “km 34 de la route de Moçâmedes à Sa da Bandeira” (= 34 km from Namibe on Lubango road) and documented by Laurent (1964). All the above specimen data are pooled in the summary tables of scalation in the revision of the *N. tessellata* complex (Broadley 1972), and he concluded that the Angolan material represents an undescribed species.

During recent surveys in south-western Angola, several individuals of *Nucras* were collected. This new material is compared with historical material of the species known from Angola and supplemented with phylogenetic analyses to investigate their taxonomic status, and to advance our understanding of the *N. tessellata* complex.

Materials and Methods

Sampling and material examined. During a recent expedition to south-western Angola, two *Nucras* individuals were collected from Namibe Province (Fig. 1). Each specimen was collected as a voucher, fixed in 10% formalin and thereafter transferred to 70% ethanol for long-term storage at the Port Elizabeth Museum (PEM). Prior to fixation, a tissue sample was collected and preserved in 99% ethanol. Material from the following museums was examined (Table 1) by Donald Broadley: Museu Bocage Lisboa, Portugal (MBL), Museu Regional do Dundo, Dundo, Angola (MD), and the British Museum (now Natural History Museum, London) [NHML]. WRB examined material in the Transvaal Museum (now Ditsong National Museum of Natural History Northern Flagship Institute, Pretoria) [TM], and re-examined and photographed the NHML specimens. Photographs of Monard’s (1937) material from the Musée d’Histoire Naturelle, La-Chaux-de-Fond, Switzerland (MHNC, formerly LCFM) were made available by Luis Ceriaco. The Angolan material was further compared to other material housed in the PEM.

Morphological data. To quantify morphology for the species diagnoses, the following measurements were recorded from each individual: snout-vent length (SVL): tip of snout to anterior edge of cloaca; tail length (Tail): tip of tail to posterior edge of cloaca; total length (TL): combined SVL and tail length; head length (HL): from anterior edge of occipital/parietal scale to tip of snout; head width (HW): width of head (just behind eye); snout length (SL): from anterior corner of eye to tip of snout; eye length (EL): horizontal diameter of eye; ear-eye length: from posterior corner of eye to anterior edge of ear opening.

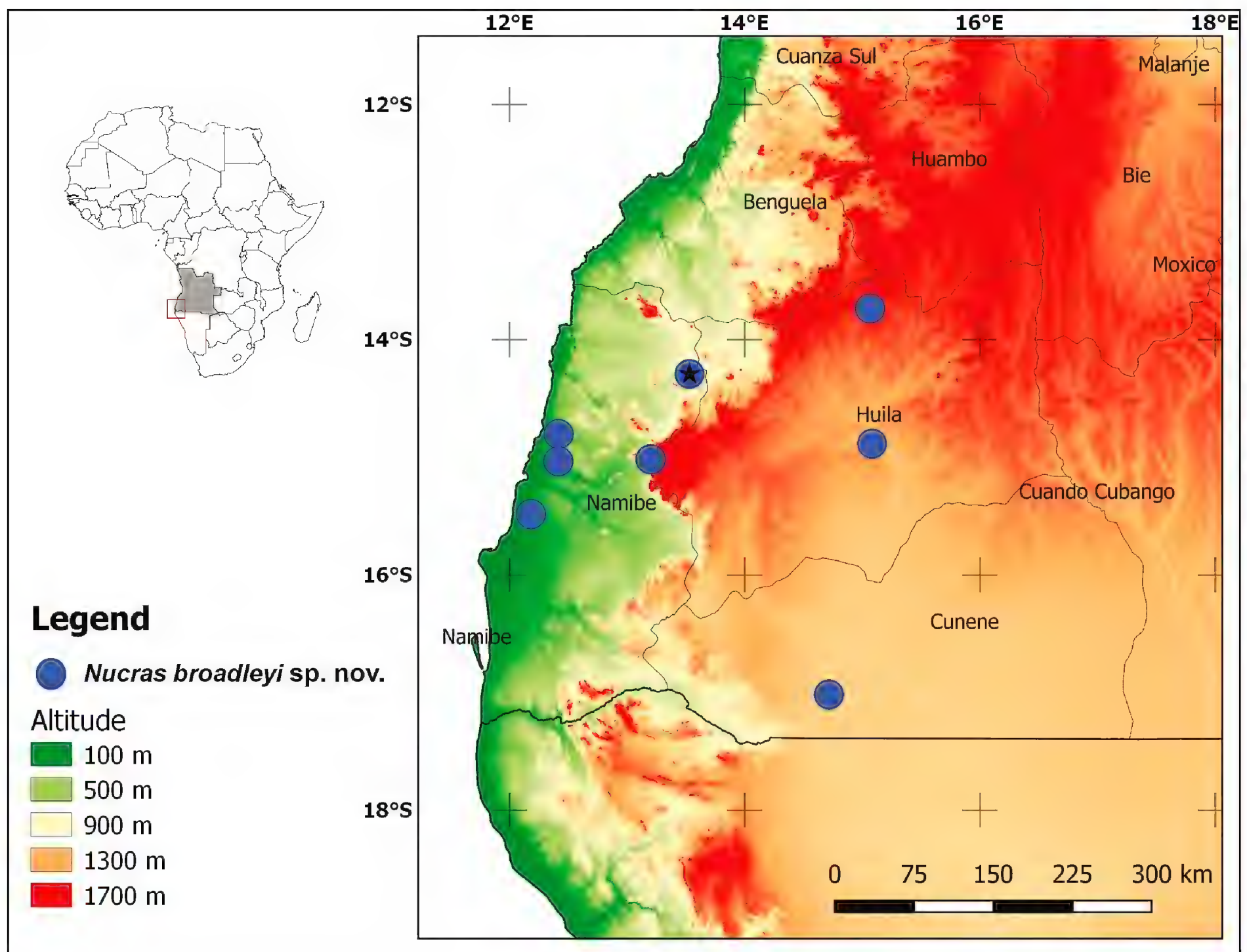


Fig.1. Map showing distribution of *Nucras broadleyi* sp. nov. in Angola.

The following scalation details were recorded: upperlabials (UL): in front of subocular and after subocular; lowerlabials (LL), transverse rows of ventrals, longitudinal ventral scale rows, supraciliars (SC), granules between supraciliars (SC) and subocular, number of subdigital lamellae below 4th toe, and number of femoral pores. All counts were performed on both left and right sides. The presence of interparietal and whether it was in contact with occipital were also recorded.

Phylogenetic analyses. To place the two *Nucras* individuals recently collected from Angola in a phylogenetic context, one nuclear (RAG-1) and two mitochondrial (ND4, 16S) genes were sequenced (Table 2). DNA was extracted using salt extraction (Aljanabi and Martinez 1997), with PCR amplification, and cycle sequencing following standard procedures. A 25 µl PCR reaction included 3 µl of 1 mM dNTPs, 3 µl of 25 mM MgCl₂, 0.2 µl of 10 pmol forward and reverse primers, 3 µl of buffer solution (20 mM Tris-HCl ~pH 8.0, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT), 0.1 µl (0.5U) Taq polymerase, and 1–2 µl of 25 ng/µl genomic DNA. Thermal cycling was run with initial denaturation for 4 min at 94 °C followed by: 35 cycles with denaturation for 30 s at 94 °C, annealing for 40 s at 55–57 °C, extension

for 40 s at 72 °C, and final extension for 4 min at 72 °C. Primers used for amplification were ND4: ND4 (Forstner et al. 1995) and Leu1 (Arévalo et al. 1994), 16S: L2510 and H3080 (Palumbi 1996); and RAG-1: RAG1-F0 and RAG1-R1 (Mayer and Pavlicev 2007). PCR products were run on a 1% agarose gel and visualized under a UV light to verify amplification. Amplicons were sequenced directly using the forward primers at Macrogen (Amsterdam, Netherlands). Sequences were edited and aligned using Geneious software v4.7 (Kearse et al. 2012). New sequences have been deposited in GenBank (Table 2). In addition, gene sequences for multiple individuals of all *Nucras* species (except *N. scalaris*) and sequences representing outgroup taxa were downloaded from GenBank (Table 2).

A Bayesian analysis of 2,052 characters from the two mitochondrial genes and one nuclear gene (ND4: 678 bp, 16S: 482 bp, RAG-1: 892 bp) was used to investigate optimal tree space using MrBayes v3.2.2 (Huelsenbeck and Ronquist 2001) at the CIPRES Science Gateway (Miller et al. 2010). To determine which evolutionary model best fit the data, jModeltest was initially run (Posada 2008). The AIC test specified the GTR+G model for both mitochondrial markers and HYK+G for RAG-1. Therefore, three unlinked data partitions were created, specifying six

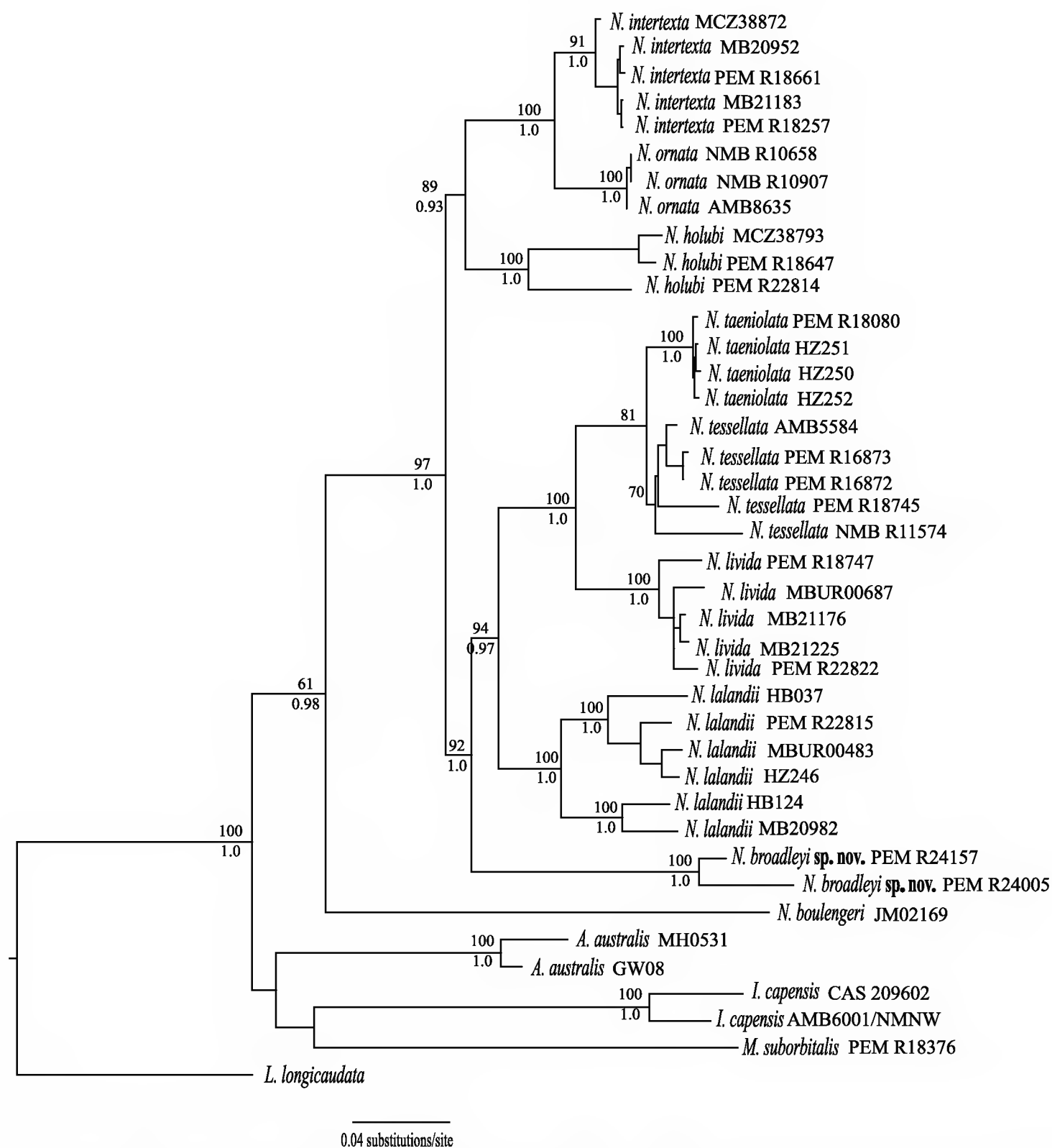


Fig. 2. Maximum likelihood topology for *Nucras* with bootstrap values (top) and Bayesian posterior probabilities (bottom) at each node. Bootstrap values <60%, posterior probabilities <0.90, and node support within each species is not shown.

(mitochondrial genes) and two (RAG-1) rate categories, including the gamma distribution, with uniform priors for all parameters. For 16S, 38 bases were excluded due to poor alignment. To ensure the robustness of results, the MCMC was run twice in parallel for 20 million generations (four chains in each run), with trees sampled every 1,000 generations. A 10% burn-in was examined (2 million generations, 2,000 trees) in Tracer v1.6 (<http://beast.bio.ed.ac.uk>) to check that the effective sample size (ESS) of all parameters met a threshold of 200 after burn-in. A 50% majority rule tree was constructed and nodes with ≥ 0.95 posterior probability were considered supported.

In addition to the Bayesian analysis, a maximum likelihood (ML) search was run using RAxML HPC 7.2.8 (Stamatakis 2006) on the CIPRES Science Gateway (http://www.phylo.org/sub_sections/portal/) for the combined dataset. The datasets were partitioned as in the Bayesian analysis, with a GTR+I+G model for all markers and 1,000 bootstrap replicates (Stamatakis et al. 2008). This analysis was run three times to ensure that independent ML searches produced the same topologies. Nodes with a bootstrap value

of $\geq 70\%$ were considered as supported in this analysis.

Pairwise sequence divergence values (uncorrected net *p*-distances) were estimated between species for both markers using MEGA v7 (Kumar et al. 2016). In addition, a barcoding approach was used to compare inter- and intra-specific sequence divergences, using SpeciesIdentifier v1.8 (Meier et al. 2006). Pairwise comparisons were generated for all *Nucras* individuals in the phylogeny for each gene, and frequency distributions of inter- and intra-specific comparisons were made. The ND4 gene was truncated 433 bp, as some GenBank sequences had only partial sequences for that gene.

Results

Phylogenetic analyses. The phylogenetic analyses show that the two individuals from Angola are in the same clade, and it is sister to a clade containing *N. livida*, *N. taeniolata*, and *N. tessellata* (Fig. 2). The new Angolan clade is well-supported by both Bayesian and likelihood analyses. Uncorrected net *p*-distances for each of the genes are

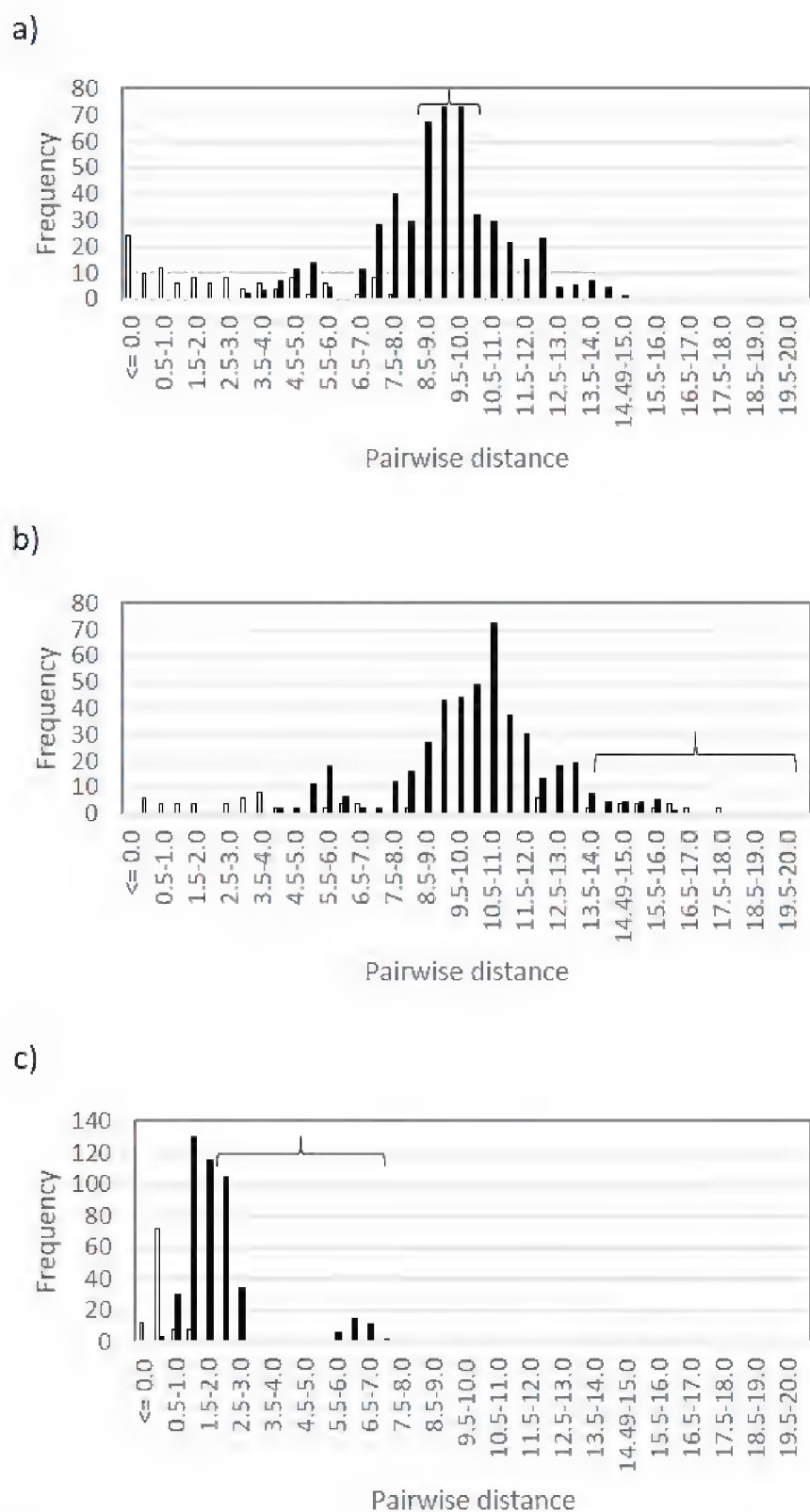


Fig. 3. Frequency distribution of pairwise sequence divergences for *Nucras* species for a) 16S, b) ND4, and c) RAG-1. Inter-specific differences shown as black bars, and intra-specific differences as white bars. The ranges of values relating *Nucras broadleyi* sp. nov. with other members of the *N. tessellata* clade are indicated by brackets.

similar to those found for other species of *Nucras* (Table 3), and the frequency distribution of pairwise differences shows that these Angolan individuals fall in the range of inter-specific divergence values (Fig. 3).

Systematics. Based on the minor morphological differences and the distinct dorsal coloration differences observed among material examined, combined with the abovementioned genetic evidence, the Angolan material is described below as a new species. No historical names are available for this clade, thus leaving no outstanding taxonomic issues (Broadley 1972; Uetz et al. 2017).

***Nucras broadleyi* sp. nov.**

Angolan Sandveld Lizard

urn:lsid:zoobank.org:act:C82E3A75-96FF-4D2A-9B52-3ABF4B58BC2B

(Figures 4–6)

Chersonomy. *Nucras tessellata* var. *taeniolata* (Bocage 1895: 30), *Nucras tessellata* var. *taeniolata* (Boulenger 1910: 474), *Nucras tessellata* var. *holubi* (Boulenger 1917: 210), *Nucras intertexta* var. *holubi* (Boulenger 1920: 20), *Nucras tessellata* (Monard 1937: 73; Laurent 1964: 56), *Nucras ornata* (Broadley 1965: 23), *Nucras tessellata* (Broadley 1972: 30; Ceriaco et al. 2016: 56; Burger 2014: 171), *Nucras* aff. *tessellata* (Marques et al. 2018: 221; Branch et al. 2019: 317).

Type material. The type series is comprised of the three most recently collected specimens, which are housed in PEM and TM.

Holotype. A subadult male (PEM R24005, AG 018), 10 km west of Lola, edge of Bentiaba River valley, Namibe Province, Angola (-14.29028, 13.53056, WGS 84, 802 m asl). Collected by W.R. Branch, P. Vaz Pinto, and J.S. de Almeida on 2 November 2015.

Paratypes (2). a) A subadult female (PEM R24157, AG 166), 8.8 km southwest of Farm Mucungo, Namibe Province, Angola (-14.80167, 12.41917, WGS 84, 385 m asl). Collected by W.R. Branch, P. Vaz Pinto, and J.S. de Almeida on 8 November 2015. b) An unsexed adult (TM 40392), “34 km S of Moçâmedes to Porto Alexandre, Angola, 1512Ca” (= 34 km S Namibe to Tômbwa), Namibe Province, Angola (approx. -15.48220, 12.18289). Collected by W.D. Haacke on 30 March 1971.

Additional referred material: The following additional material was used to expand the description of variation within the species: a) an adult male (MD 1967, Laurent 1964), “km 34 de la route de Moçâmedes à Sa da Bandeira” (=34 km from Namibe on Lubango road, -15.03333, 12.41667), collected 24 October 1949, b) MBL 646, 647a, 647b (Bocage 1895: 30) from Maconjo (approx. -15.01667, 13.20000), c) BM 1970.6.29.10–11 (Boulenger 1910: 474) from Ponang Kuma (= Donguena, approx. -17.01667, 14.71667), and d) MHNC 91.0524 (Monard 1937) from Capelongo (approx. -14.88333, 15.08333), collected April 1933.

Etymology. The specific epithet is a patronym in honor of Donald G. Broadley for his numerous contributions to the herpetofauna of Africa. Don (as most of us knew him) was the first to recognize the Angolan population as a separate species (Broadley 1972). The name is constructed in the masculine genitive.

Diagnosis. Assigned to *Nucras* due to a well-defined collar (absent in *Ichnotropis*), toes not serrated or fringed (versus serrated or fringed in *Meroles*), subdigital lamellae smooth (versus keeled in *Pedioplanis* and *Heliobolus*), subocular

Table 1. Meristic and scalation data for Angolan *Nucras broadleyi* sp. nov. PEM – Port Elizabeth Museum, BM – Natural History Museum, London (formerly British Museum), MD – Museu Dundo Regional do Dundo, Angola, TM – Ditsong Museum of Natural History, South Africa (formerly Transvaal Museum), MBL – Museu Bocage Lisboa, Portugal (material destroyed), MHNC – Musée d' Histoire Naturelle de La Chaux-de-Fonds, Chaux-de-Fonds, Switzerland.

Museum number	PEM R24005	PEM R24157	TM 40397	MD 1967	MBL 646	MBL 647a	MBL 647b	BM 1907.6.29.10	BM 1907.6.29.11	MHNC 91.0524–5
Status	Holotype	Paratype	Paratype	Additional specimen	Additional specimen	Additional specimen	Additional specimen	Additional specimen	Additional specimen	Additional specimens
Sex	Male	Female	Male	Male						Juveniles
SVL	36	44	58	40 (39)	57	63	52	74	60	
Tail	t	t	110	90 (91)	120+	133	135	144+	114	
Total	-	-	168	130	177+	193	187	218	174	
Head length (HL)	10.5	9.7	12.4	9.5	12.1	13.8	12.8			
Head width (HW)	5.88	6.4	6.2							
HW/HL ratio	0.56	0.7	0.5							
Snout length	3.8	4.1	4.9							
Eye length	2.11	2.1	2.4							
Ear-eye length	3.74	3.5	4.2							
UL *	4 (2)	4 (2)	4 (2)					4 (2)	4 (2)	
LL **	6 (3)	6 (3)	6 (3)					6 (3)	6 (3)	
MSR	42	48	46	48 (50)	38	44	46	48	48	42–43
Ventrals (transverse)	8	8	8	8	8	8	8	8	8	
Ventrals (longitudinal)	28	29	27	29	33	30	29	33	29	27–29
Granules SC-SO	1/0	3/4	2/3	3	1	2	6/3	3/2	3/3	0–2
Supraciliaries	6/6	7/8	6/6	6	5	6	7	7/6	6	
Interparietal Y/N	Y	Y	Y	Y				Y	Y	
IP contact with occipital	contact	contact	contact	contact	separate	broad contact	broad contact	contact	contact	
Subdigital lamellae below 4 th toe	23/25	27/27	23/25	28/29 (27)	27/26	28/27	28/28	28/28	26/26	25
Femoral pores	13/13	15/15	14/16	13/13	14/13	12/12	15/14	14/14	13/13	

* anterior subocular (posterior subocular), ** total (largest number, t = truncated)

bordering lip, the nostril is pierced between two nasals, nasal well separated from upper labial, and dorsal scales small, smooth, and juxtaposed.

The new species can be diagnosed from other *Nucras* species based on a combination of the following characters: series of transversely enlarged plates present under forearm (versus absent or only feebly enlarged in *Nucras lalandii*), a small series (0–6) of small granules present between

supraciliaries and supraoculars (versus mostly absent in *N. boulengeri* and *N. lalandii*), 23–29 lamellae under 4th toe (versus less than 22 in *N. lalandii*), dorsum with a series of longitudinal pale stripes (versus dark cross bands present in *N. lalandii* and *N. scalaris* or a series of pale vertebral spots, sometimes forming irregular transverse bands in *N. intertexta* or lack of any dorsal patterns in *N. aurantiaca*), four pale stripes on nape with outer stripes forming a

Table 2. Samples used in genetic analysis. Museum abbreviations: CAS – California Academy of Science, PEM – Port Elizabeth Museum, NMB – National Museum Bloemfontein.

Genus	Species	Field accession ID	Museum accession number	16S	ND4	RAG1	Locality
<i>Nucras</i>	<i>broadleyi</i> sp. nov.	AG18	PEM R24005	MN265869y	—	MN265872y	Angola, Namibe Province
<i>Nucras</i>	<i>broadleyi</i> sp. nov.	AG166	PEM R24157	MN265870y	MN265871y	MN265873y	Angola, Namibe Province
<i>Nucras</i>	<i>boulengeri</i>	JM02169		HG005184	HG005212	HG005233	Kenya
<i>Nucras</i>	<i>holubi</i>	MBUR00260	PEM R22814	HG005188	HG005216	HG005237	South Africa, Limpopo Province
<i>Nucras</i>	<i>holubi</i>	MCZ38793	CAS 234138	HG005186	HG005214	HG005235	South Africa, Limpopo Province
<i>Nucras</i>	<i>holubi</i>	RSP420	PEM R18647	HG005187	HG005215	HG005236	South Africa, Limpopo Province
<i>Nucras</i>	<i>intertexta</i>	MB20952		HG005193	HG005221	HG005242	South Africa, Northern Cape Province
<i>Nucras</i>	<i>intertexta</i>	MB21183		HG005194	HG005222	—	South Africa, Northern Cape Province
<i>Nucras</i>	<i>intertexta</i>	MCZ38872	CAS 234212	HG005192	HG005220	HG005241	South Africa, Limpopo Province
<i>Nucras</i>	<i>intertexta</i>	RSP030	PEM R18257	HG005191	HG005219	HG005240	South Africa, Northern Cape Province
<i>Nucras</i>	<i>intertexta</i>	RSP277	PEM R18661	HG005190	HG005218	HG005239	South Africa, Northern Cape Province
<i>Nucras</i>	<i>lalandii</i>	HB037		HF951554	HF951533	HF951538	South Africa, KwaZulu-Natal Province
<i>Nucras</i>	<i>lalandii</i>	HB124		HF951553	HF951532	HF951537	South Africa, Eastern Cape Province
<i>Nucras</i>	<i>lalandii</i>	HZ246		HF951555	HF951534	HF951539	South Africa, Eastern Cape Province
<i>Nucras</i>	<i>lalandii</i>	MB20982		HG005197	HG005225	HG005245	South Africa, Western Cape Province
<i>Nucras</i>	<i>lalandii</i>	MBUR00414	PEM R22815	HG005195	HG005223	HG005243	South Africa, Eastern Cape Province
<i>Nucras</i>	<i>lalandii</i>	MBUR00483		HG005196	HG005224	HG005244	South Africa, Eastern Cape Province
<i>Nucras</i>	<i>livida</i>	KTH08-071	PEM R18747	HG005200	HG005227	HG005247	South Africa, Western Cape Province
<i>Nucras</i>	<i>livida</i>	MB21176		HG005201	HG005228	HG005248	South Africa, Northern Cape Province
<i>Nucras</i>	<i>livida</i>	MB21225		HG005202	HG005229	HG005249	South Africa, Northern Cape Province
<i>Nucras</i>	<i>livida</i>	MBUR00670	PEM R22822	HG005198	—	HG005246	South Africa, Eastern Cape Province
<i>Nucras</i>	<i>livida</i>	MBUR00687		HG005199	HG005226	—	South Africa, Western Cape Province
<i>Nucras</i>	<i>ornata</i>	AMB8635	PEM R17591	HG005206	—	HG005252	South Africa, Limpopo Province
<i>Nucras</i>	<i>ornata</i>	MBUR01226	NMB R10907	HG005203	—	HG005250	South Africa, KwaZulu-Natal Province
<i>Nucras</i>	<i>ornata</i>	MBUR01230	NMB R10658	HG005204	—	HG005251	South Africa, KwaZulu-Natal Province
<i>Nucras</i>	<i>taeniolata</i>	HZ250		HG005207	—	HG005253	South Africa, Eastern Cape Province
<i>Nucras</i>	<i>taeniolata</i>	HZ251		HG005208	HG005230	HG005254	South Africa, Eastern Cape Province
<i>Nucras</i>	<i>taeniolata</i>	HZ252		HG005209	—	HG005255	South Africa, Eastern Cape Province
<i>Nucras</i>	<i>taeniolata</i>	PEM R18080	PEM R18080	HG005210	HG005231	HG005256	South Africa, Eastern Cape Province

Table 2 (continued). Samples used in genetic analysis. Museum abbreviations: CAS – California Academy of Science, PEM – Port Elizabeth Museum, NMB – National Museum Bloemfontein.

Genus	Species	Field accession ID	Museum accession number	16S	ND4	RAG1	Locality
<i>Nucras</i>	<i>tessellata</i>	AMB5584	CAS 206725	HG005211	HG005232	HG005257	South Africa, Northern Cape Province
<i>Nucras</i>	<i>tessellata</i>	KTH08-069	PEM R18745	HF951559	—	HF951543	South Africa, Western Cape Province
<i>Nucras</i>	<i>tessellata</i>	MB20650	PEM R16873	HF951556	HF951535	HF951540	South Africa, Northern Cape Province
<i>Nucras</i>	<i>tessellata</i>	MB21061	NMB R11574	HF951558	—	HF951542	South Africa, Northern Cape Province
<i>Nucras</i>	<i>tessellata</i>	MB20687	PEM R16872	HF951557	HF951536	HF951541	South Africa, Northern Cape Province
Outgroup							
<i>Latastia</i>	<i>longicaudata</i>			AF080358	—	EF632229	GenBank
<i>Australolacerta</i>	<i>australis</i>	MH0531	NA	DQ871152	FR751398	DQ871208	South Africa, Western Cape Province
<i>Australolacerta</i>	<i>australis</i>	GW08	NA	HF547772	HF547725	HF547691	South Africa, Western Cape Province
<i>Ichnotropis</i>	<i>capensis</i>	AMB6001	NMNW	DQ871148	HF547732	DQ871206	Namibia, Kamanjab
<i>Ichnotropis</i>	<i>capensis</i>	AMB6067	CAS 209602	DQ871149	HF547733	DQ871207	South Africa, KwaZulu-Natal Province
<i>Meroles</i>	<i>suborbitalis</i>	SVN049	PEM R18376	HF547800	HF547759	HF547718	South Africa, Western Cape Province

continuous light stripe with the outer edges of the parietals (similar to Broadley’s (1972) *N. tessellata tessellata* var. “T;” differs from *N. livida* and *N. tessellata* where the outer stripes often do not form a continuous light stripe with the outer edges of the parietals; differs from *N. caeiscaudata* and *N. ornata* where there are only three longitudinal stripes present on nape and sometimes the vertebral ones are absent), well defined occipital scale separating parietals (versus reduced or absent in northern Namibia *N. holubi*, which is referred to as *N. intertexta damarana* Parker; as well as absent in *N. caeiscaudata*), parietal foramen absent (often present in all other species except *N. taeniolata*), and postnasals separated (usually fused in *N. taeniolata*).

In the phylogenetic analysis, the uncorrected *p*-distances show that this clade differs by >8% for 16S, >14% for ND4, and >1% for RAG1 sequence divergence from other members of the *N. tessellata* clade.

Description of Holotype (Fig. 4). Body relatively slender (SVL approx. 4.5 times the head length, tail truncated), with hindlimbs larger than forelimbs (femur of hind limb equal to length of tibia); head narrow and elongated (56% longer than wide) with narrow pointed but blunt snout, that is slightly longer than distance from back of eye to rear of ear opening. Rostrum protruding and visible from below. Nasals paired and in contact (0.2 mm suture length), not swollen, nostril directed backwards separating postnasals. Frontonasal single, wider than long (1.1 × 1.8 mm). Prefrontals paired and in broad median contact with one another (0.6 mm suture length), wider than long (1.1 × 1.2 mm). Frontal entire, longer than wide (2.7 × 1.9 mm). Two large rounded supraoculars, both in contact with the frontal, with anterior supraocular preceded by a single large scale in contact with prefrontal, frontonasal, and posterior loreal, with posterior supraocular bordered by a single large scale in contact with parietal and frontoparietal. Paired frontoparietal in broad contact (1.3 mm suture length), nearly as wide as long (1.7 × 1.5 mm). Parietals twice as long as wide (3.1 × 1.8 mm), fully separate by a large, pentagonal interparietal (2.5 × 1.2 mm) that is twice as long as wide, slightly shorter than frontoparietals and nearly equal to length of frontonasal and prefrontal combined. Small subtriangular occipital (0.5 × 0.7 mm). Two loreals, second much larger than first. Six supraciliaries on each side, 1st is the longest. A single minute granule scale between supraocular and supraciliaries on right side, none on left side. Four supralabials anterior to subocular and three supralabials posterior to subocular, on both sides. Subocular slightly elevated medial and bordering the lip, its lower border being shorter than the upper. Three temporal scales, first longer than others, smooth. Tympanic shield as wide as long, border of ear opening. No ear lobes. Lower eyelid with transparent brille formed by five larger scales, surrounded by numerous smaller scales. Lower eyelid separated from subocular and enlarged temporal scales by a series of 10 smaller scales. Small scale above 3rd supralabial separating the posterior loreal and subocular.

A new *Nucras* species from Angola

Table 3. Pairwise uncorrected net *p*-distances for species of *Nucras*: a) 16S, b) ND4, c) RAG-1. Comparisons not made due to missing data indicated by *na*.

a)	1	2	3	4	5	6	7	8	9
1 <i>broadleyi</i> sp. nov	0.048								
2 <i>lalandii</i>	0.083	0.047							
3 <i>livida</i>	0.097	0.058	0.019						
4 <i>taeniolata</i>	0.098	0.056	0.084	0.000					
5 <i>tessellata</i>	0.075	0.043	0.026	0.073	0.027				
6 <i>boulengeri</i>	0.105	0.101	0.117	0.116	0.103	<i>na</i>			
7 <i>holubi</i>	0.074	0.044	0.066	0.063	0.053	0.079	0.048		
8 <i>intertexta</i>	0.084	0.068	0.090	0.085	0.078	0.105	0.043	0.008	
9 <i>ornata</i>	0.083	0.067	0.091	0.082	0.076	0.108	0.050	0.043	0.000
b)									
1 <i>broadleyi</i> sp. nov	<i>na</i>								
2 <i>lalandii</i>	0.139	0.124							
3 <i>livida</i>	0.194	0.115	0.045						
4 <i>taeniolata</i>	0.208	0.147	0.121	0.003					
5 <i>tessellata</i>	0.198	0.138	0.112	0.006	0.018				
6 <i>boulengeri</i>	0.219	0.188	0.233	0.277	0.267	<i>na</i>			
7 <i>holubi</i>	0.153	0.092	0.137	0.156	0.146	0.199	0.115		
8 <i>intertexta</i>	0.208	0.141	0.175	0.196	0.193	0.239	0.118	0.017	
c)									
1 <i>broadleyi</i> sp. nov	0.001								
2 <i>lalandii</i>	0.012	0.002							
3 <i>livida</i>	0.014	0.009	0.004						
4 <i>taeniolata</i>	0.013	0.007	0.009	0.004					
5 <i>tessellata</i>	0.012	0.006	0.008	0.007	0.008				
6 <i>boulengeri</i>	0.066	0.059	0.064	0.064	0.058	<i>na</i>			
7 <i>holubi</i>	0.020	0.015	0.017	0.016	0.016	0.068	0.004		
8 <i>intertexta</i>	0.017	0.012	0.013	0.012	0.013	0.064	0.015	0.001	
9 <i>ornata</i>	0.021	0.016	0.016	0.016	0.017	0.064	0.019	0.004	0.001

Enlarged scale bordering 1st post subocular, supralabial, and the subocular. Six infralabials on both sides, with 3rd being longest; four enlarged pairs of chin shields, last largest and first three in broad contact. Twenty-four gular scales in a straight line between symphysis of chin shields and median collar plate, equal in size except last 4–5 larger. Collar free, comprising seven enlarged plates (median subtriangular) and extending slightly onto side of neck as a crease, bordered by 2–3 smaller scales. Dorsal scales small, juxtaposed, granular, smooth, larger on sides toward ventrals. Midbody scales 42. Ventral plates eight longitudinal and 28 transverse rows (from collar to groin), plates of the innermost rows longer than broad, with outer row notably smaller than other rows, transverse row of ventrals across chest just behind collar longer than broad; preanal scales irregular, median ones larger. Scales on upper surface of forearm large, smooth or slightly keeled. Scales on lower surface of forearm with eight enlarged plates, at least twice the width of scales on upper forearm.

Scales on upper surface of tibia rhombic, subimbricate, smooth, and much larger than dorsal scales. Tibia below with a series of large plates. Subdigital lamellae under fourth toe 23R/25L. Femoral pores 13R/15L. Dorsal scales on tail oblique, strongly keeled diagonally, and truncate behind, ventral scales on tail obtusely keeled.

Coloration. Dorsum with eight pale cream to white dorsolateral longitudinal stripes, separated by dark brown to black stripes. These stripes are more boldly patterned anteriorly, fading posteriorly. No light vertebral stripe. The two pale paravertebral stripes are separated by a very narrow strip of darker scales that starts on the interparietal through the occipital scale and fades posteriorly onto body and tail. The dorsolateral stripe extending along outer borders of parietals continues onto the tail. It is followed by the upper lateral stripe extending from posterior of the eye onto the head through the mid-temporal with a brief break above the ear opening, and continues onto the tail. The lower lateral stripe starts at the subocular, through the

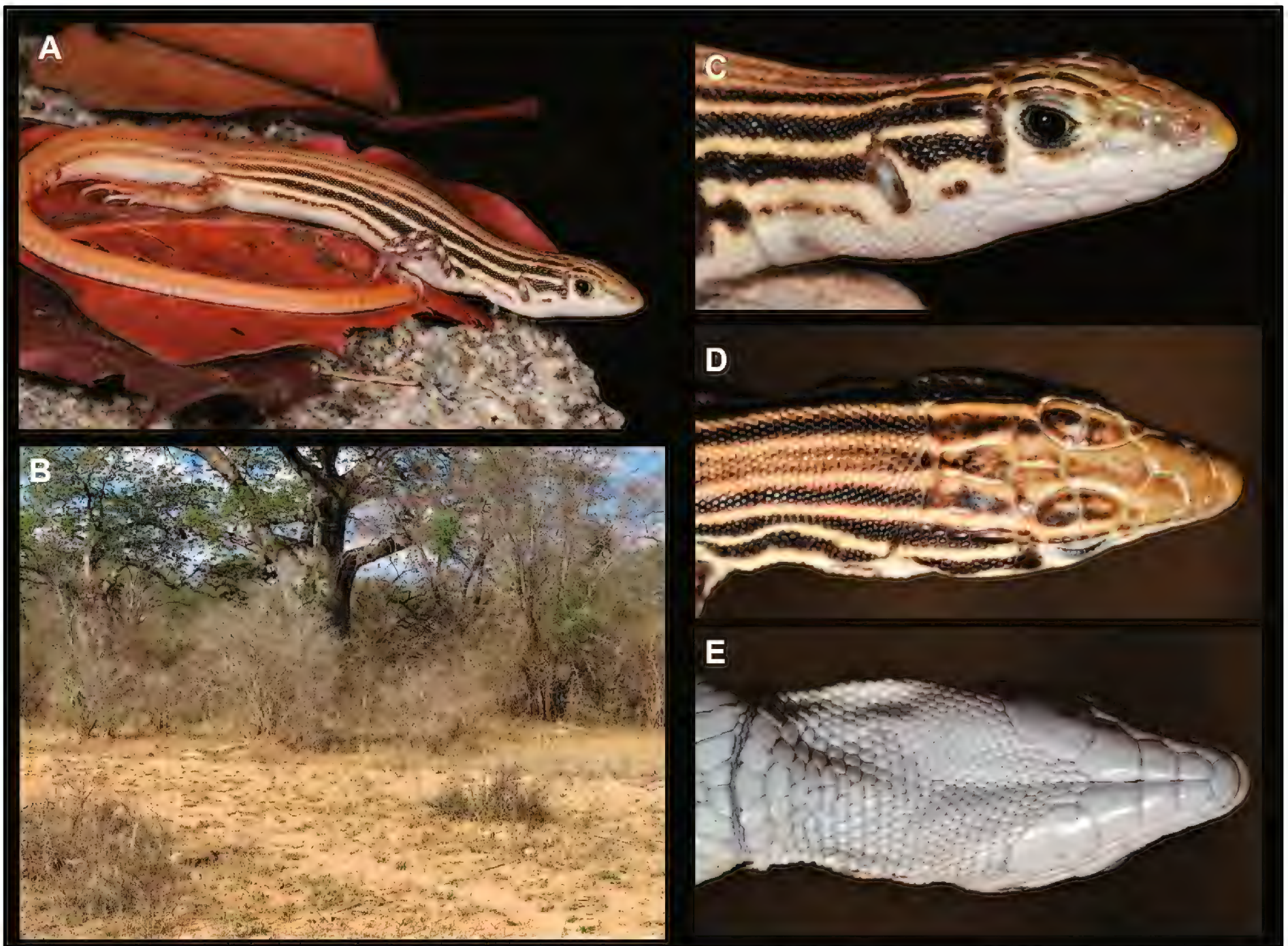


Fig. 4. *Nucras broadleyi* sp. nov. A – holotype, adult male, PEM R24005 (AG 18) in life; B – general habitat photo of type locality, 10 km west of Lola, edge of Bentiaba River valley, Namibe Province, Angola; C – lateral close-up of head of holotype; D – dorsal close-up of head of holotype; E – ventral close-up of head of holotype (Photos: Bill Branch).

ear opening, broken briefly above the arm, after which it continues all the way onto the tail. Ventrums white and lower limbs oblique white. Fore limbs upper surface black with scattered pale blotches. Hind limbs light brown with pale blotches. Upper surface of tail red-brown, similar to hind limbs. Scales bordering the orbit are black edged.

Variation (Figs. 5–6). Meristic and escalation data are summarized in Table 1. The largest specimen examined is (BM 1907.6.29.10) 74 +144 mm (tail regenerated). Regarding coloration, there seem to be three main variations among material examined: 1) 8–9 longitudinal stripes as in holotype (in PEM R24005, MBL 647a, 647b, MHNC 91.0524–5), 2) 4–5 pale longitudinal stripes broken up posteriorly with flanks spotted (in BM 1970.6.29.10–11, TM 40392, MD 1967), and 3) broken paravertebral stripes, continuous dorsolateral line and barred flanks (in PEM R24157), similar to *N. intertexta*.

Distribution. Found only in semi-arid south-western Angola, throughout much of Namibe Province and extending onto the escarpment of southern Huíla and Cunene Provinces (Fig. 1). Known localities include: Maconjo (Bocage 1895: 30), Ponang Kuma (=Donguena)

(Boulenger 1910: 472), 34 km from Namibe on Lubango road (Laurent 1964: 56), 34 km south of Tombwa (TM 40397), 8.8 km southwest of Farm Mucungo (this study), 10 km west of Lola (this study), and Capelongo (Monard 1937: 73). The locality of Caconda (Bocage 1895) extends the species distribution further north into Huíla Province, but the specimens could not be critically evaluated by Broadley (1972) and are now presumably lost.

Habitat. The species appears to be associated with mopane woodlands, dry savannas, and semi-desert shrublands (Barbosa 1970). The new material was found in sandy plains with scattered low granite outcrops, with varying degrees of short grass cover and scattered bushes. Vegetation included *Colophospermum mopane*, *Ficus* sp., *Senegalia* (=Acacia) *mellifera*, *Commiphora* sp., *Boscia foetida*, and *Salvadora persica*. The confirmed historical records were also obtained within the dry woodland zone, even though the possible occurrence of the species in Caconda would place the species above 1,500 m asl and well into the mesic conditions of *Brachystegia* habitats (Barbosa 1970).

Conservation. Population estimates for the species

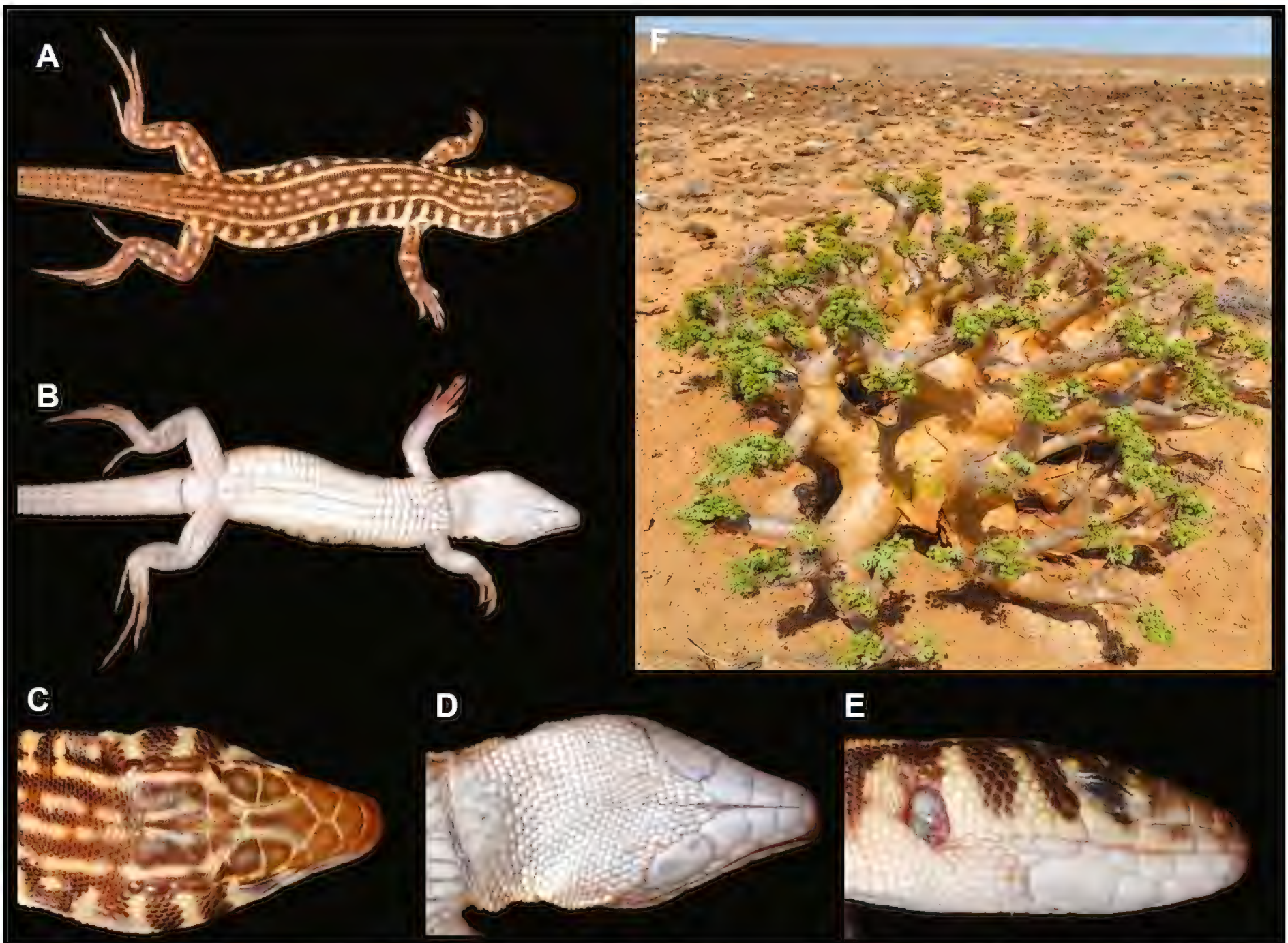


Fig. 5. *Nucras broadleyi* sp. nov. A – paratype, adult female, PEM R24157 (AG 166) dorsal view; B – ventral view; C – dorsal close-up of head of paratype; D – ventral close-up of head of paratype; E – lateral close-up of head of paratype; F – general habitat photo of type locality, 8.8 km southwest of Farm Mucongo, Namibe Province, Angola (Photos: Bill Branch).

are unknown, and only few scattered specimens (~12) are known, of which four specimens were destroyed in the Museu Bocage Lisboa fire and one of the Monard specimens is unaccounted for. However, Sandveld Lizards are secretive and less conspicuous than many other lacertids, so additional surveys are required to determine the full range of the species and to identify potential habitat threats in order to accurately assess its conservation status.

Discussion

Broadley (1972) was the first to suggest the Angolan population of *Nucras tessellata* to be different from other described species, but took no taxonomic action. Here, we present evidence to support his assumptions and formally describe the Angolan population as a new species. Thus, Angola now has two endemic species of *Nucras* and the genus now comprises 12 recognized species. As our phylogeny is built on the work of Edwards et al. (2013) we retrieved the same general topology, except for the inclusion of the new species. Although different samples and genetic markers were used, Bauer et al. (2019) retrieved the same species relationships except for the inclusion of their newly described species, *N. aurantiaca*. Thus, we can conclude that the current species relationships are

well resolved. Due to the secretive nature of members of this genus, disjunct distribution, and previously recognized varieties (see Broadley 1972), it is possible that there are other undiscovered species, particularly in areas that remain poorly surveyed.

The species appears restricted to the arid biomes of southwestern Angola at relatively low to moderate altitudes, while the records from Caconda remain problematic and may have been misidentified or incorrectly labelled. In recent years, the number of endemic species described from the arid south-western Angola has increased, e.g., *Kolekanus plumicaudus* (Haacke 2008), *Pedioplanis huntleyi* and *P. haackei* (Conradie et al. 2012), *Cordylus namakuiyus* (Stanley et al. 2016), *Cordylus phonolithos* (Marques et al. 2019b), *Poyntophrynus pachnodes* (Ceríaco et al. 2018), and now *Nucras broadleyi* sp. nov. This region also harbors numerous other endemic species, such as *Afrogecko ansorgii*, *Pachydactylus angolensis*, *Poyntonophrynus grandisonae*, *Pedioplanis benguellensis*, *Rhoptropus taeniostictus*, *Typhlacontias rudebecki*, and *T. punctatissimus bogerti* (Ceríaco et al. 2016, 2018; Marques et al. 2018; Branch et al. 2019). The growing body of information suggests there could be a unique and diverse endemic Angolan-Namib reptile fauna (Ceríaco et al. 2016; Marques et al. 2018; Branch

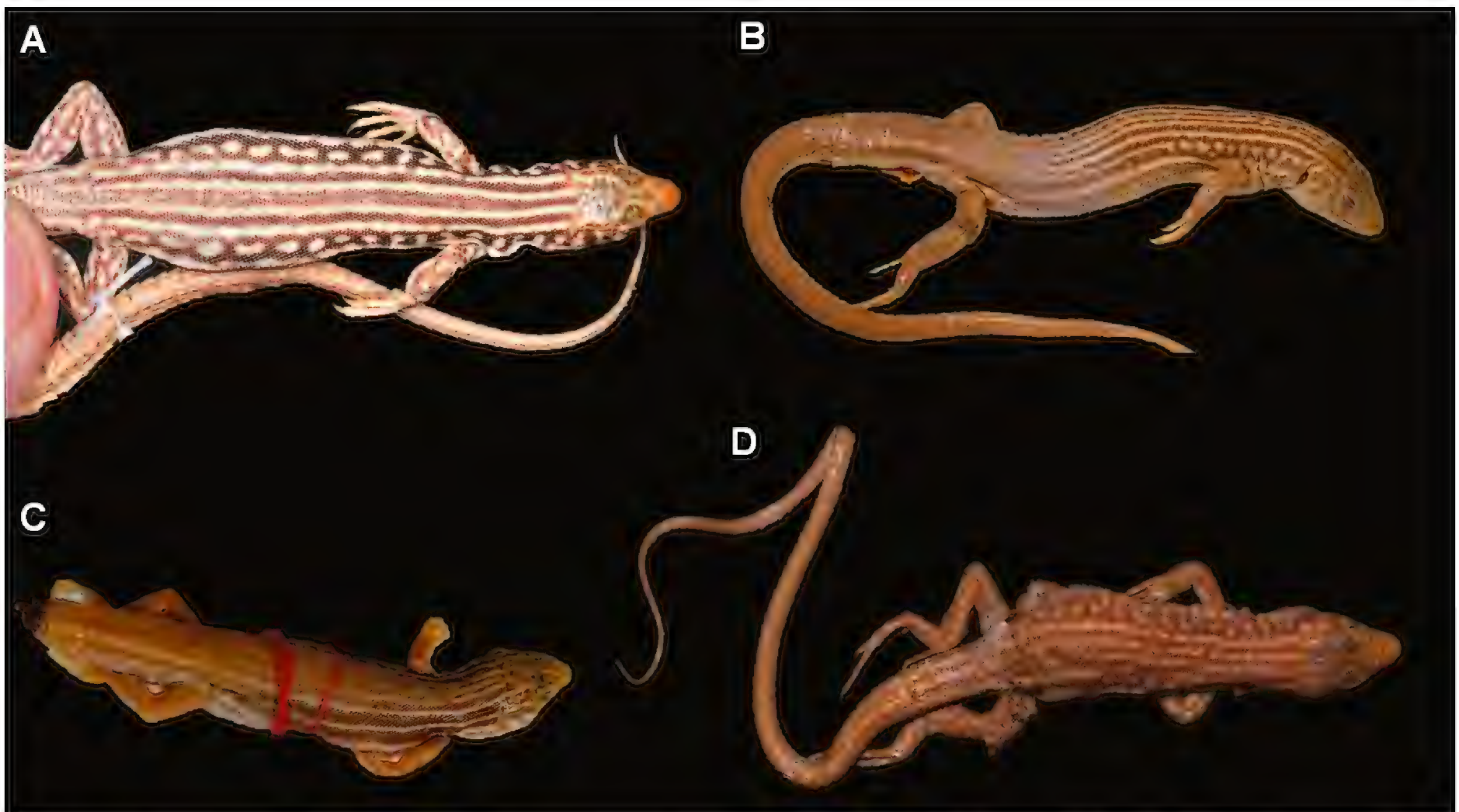


Fig. 6. Variation of *Nucras broadleyi* nov. sp. dorsal color pattern. A – TM 40392 from “34 km S of Moçâmedes to Porto Alexandre;” B – BM 1970.6.29.10 from Ponang Kuma (=Donquena); C – MHNC 91.0524 from Capelongo; D – MD 1967 from “km 34 de la route de Moçâmedes à Sa da Bandeira” (Photos: A, B – Bill Branch, C, D – Luis Ceríaco).

et al. 2019), with additional discoveries yet to be made.

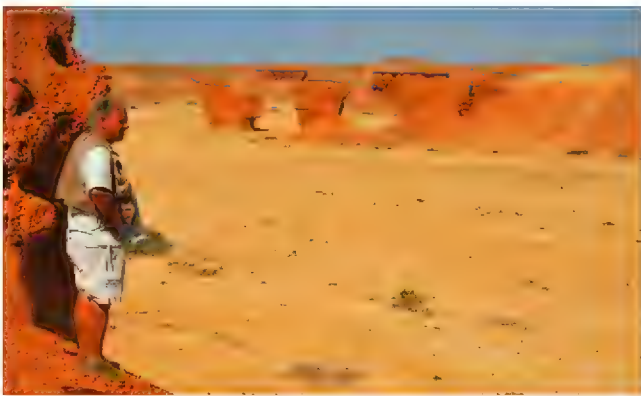
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Literature Cited

- Aljanabi SM, Martinez I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR based techniques. *Nucleic Acids Research* 25: 4,692–4,693.
- Arévalo E, Davis SK, Sites JJW. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Systematic Biology* 43: 387–418.
- Barbosa LAG. 1970. *Carta Fitogeográfica de Angola*. Instituto de Investigação Científica de Angola, Luanda, Angola. 323 p.
- Bauer AM, Childers JL, Broeckenhoven C, Mouton PLN. 2019. A new *Nucras* Gray, 1838 (Squamata: Lacertidae) from the Strandveld of the Western Cape, South Africa. *Zootaxa* 4560(1): 149–163.
- Bocage JVB. 1895. *Herpétologie d'Angola et du Congo*. Ministério da Marinha e das Colónias, Lisbonne, Portugal. 203 p., 20 pls.
- Boulenger GA. 1910. A revised list of South African reptiles and batrachians, with synoptic tables, special reference of specimens in the South African Museum, and descriptions of new species. *Annals of the South African Museum* 5: 455–538.
- Boulenger GA. 1917. A revision of the lizards of the genus *Nucras*, Gray. *Annals of the South African Museum* 13: 195–216.
- Boulenger GA. 1920. *Monograph of the Lacertidae*. Volume 1. Trustees of the British Museum (Natural History), London, United Kingdom. x + 352 p.
- Branch WR, Bauer AM. 1995. The herpetofauna of the Little Karoo, Western Cape, South Africa, with notes on life history and taxonomy. *Herpetological Natural History* 3: 47–89.
- Branch WR, Tolley KA. 2017. Oral presentation (Abstract). New lacertids from Angola. *African Herp News* 66: 11.
- Branch WR. 1998. *Field Guide to the Snakes and other Reptiles of Southern Africa*. Struik, Cape Town, South Africa. 368 p.

- Branch WR. 2016. Preface, Amphibian & Reptile Conservation special Angola-Africa issue. *Amphibian & Reptile Conservation* 10(2): i–iii (e128).
- Branch WR, Haacke W, Vaz Pinto P, Conradie W, Baptista N, Verburgt L. 2017. Loveridge's Angolan geckos, *Afroedura karroica bogerti* and *Pachydactylus scutatus angolensis* (Sauria, Gekkonidae): new distribution records, comments on type localities, and taxonomic status. *Zoosystematics and Evolution* 93: 157–166.
- Branch WR, Baptista N, Vaz Pinto P, Conradie W. 2019. The reptiles of Angola – History, updated checklists, endemism, hot spots, and future directions for research. Pp. 283–326 In: *Biodiversity of Angola. Science and Conservation: A Modern Synthesis*. Editors, Huntley BJ, Ferrand N, Russo V, Lages F. Springer Open, Cham, Switzerland. 552 p.
- Broadley DG. 1965. Some problems presented by sand lizards of the *Nucras tessellata* group. *Journal of the Herpetological Association of Africa* 1: 18–23.
- Broadley DG. 1972. A review of the *Nucras tessellata* group (Sauria: Lacertidae). *Arnoldia* 20: 1–35.
- Burger M. 2014. *Nucras tessellata* (A. Smith, 1838). Pp. 171–172 In: *Atlas and Red List of the Reptiles of South Africa, Lesotho, and Swaziland*. Editors, Bates MF, Branch WR, Bauer AM, Burger M, Marais J, Alexander GJ, de Villiers MS. Suricata 1. South African National Biodiversity Institute, Pretoria, South Africa. 485 p.
- Ceríaco LMP, Marques MP, Bandeira S, Agarwal I, Stanley EL, Bauer AM, Heinicke MP, Blackburn DC. 2018. A new earless species of *Poyntonophrynus* (Anura, Bufonidae) from the Serra da Neve Inselberg, Namibe Province, Angola. *ZooKeys* 780: 109–136.
- Ceríaco LMP, de Sá SC, Bandeira S, Valério H, Stanley EL, Kuhn AL, Marques M, Vindum JV, Blackburn DC, Bauer AM. 2016. Herpetological survey of Iona National Park and Namibe Regional Natural Park, with a synoptic list of the amphibians and reptiles of Namibe Province, Southwestern Angola. *Proceedings of the California Academy of Sciences* 63(2): 15–61.
- Conradie W, Branch WR, Measey GJ, Tolley KA. 2012. Revised phylogeny of Sand lizards (*Pedioplanis*) and the description of two new species from south-western Angola. *African Journal of Herpetology* 60: 91–112.
- FitzSimons VFM. 1943. The lizards of South Africa. *Memoirs of the Transvaal Museum* 1: 1–528.
- Forstner MRJ, Davis SK, Arevalo E. 1995. Support for the hypothesis of Anguimorph ancestry for the suborder Serpentes from phylogenetic analysis of mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 4: 93–102.
- Haacke WD. 2008. A new leaf-toed gecko (Reptilia: Gekkonidae) from south-western Angola. *African Journal Herpetology* 57(2): 85–92.
- Haagner GV, Branch WR, Haagner AJF. 2000. Notes on a collection of reptiles from Zambia and adjacent areas of the Democratic Republic of the Congo. *Annals of Amphib. Reptile Conserv.*
- the Eastern Cape Museum* 1: 1–25.
- Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
- Jacobsen NHG. 1989. *A Herpetological Survey of the Transvaal*. Ph.D. Dissertation, University of Natal, Department of Biological Sciences, Durban, South Africa. 1,621 p.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12): 1,647–1,649.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1,870–1,874.
- Laurent RF. 1964. Reptiles et amphibiens de l'Angola (troisième contribution). *Publicações Culturais Companhia de Diamantes de Angola* 67: 1–165.
- Marques MP, Ceríaco LMP, Bandeira S, Pauwels OSG, Bauer AM. 2019a. Description of a new long-tailed skink (Scincidae: *Trachylepis*) from Angola and the Democratic Republic of the Congo. *Zootaxa* 4568(1): 51–68.
- Marques MP, Ceríaco LMP, Stanley EL, Bandeira S, Agarwal I, Bauer AM. 2019b. A new species of Girdled Lizard (Squamata: Cordylidae) from the Serra da Neve Inselberg, Namibe Province, southwestern Angola. *Zootaxa* 4668(4): 503–524.
- Marques MP, Ceríaco LMP, Blackburn DC, Bauer AM. 2018. Diversity and distribution of the amphibians and terrestrial reptiles of Angola: atlas of historical and bibliographic records (1840–2017). *Proceedings of the California Academy of Sciences Series 4*, 65(Supplement II): 1–501.
- Mayer W, Pavlicev M. 2007. The phylogeny of the family Lacertidae (Reptilia) based on nuclear DNA sequences: convergent adaptations to arid habitats within the subfamily Eremiainae. *Molecular Phylogenetics and Evolution* 44: 1,155–1,163.
- Meier R, Kwong S, Vaidya G, Ng PKL. 2006. DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology* 55: 715–728.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 In: *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, Louisiana, USA*. Institute of Electrical and Electronics Engineers (IEEE), Piscataway, New Jersey, USA. 115 p.
- Monard A. 1937. Contribution à l'herpétologie d'Angola. *Arquivos do Museu Bocage* 8: 19–154.
- Neumann O. 1900. Description of a new lizard of the genus *Nucras* from Usoga, British East Africa. *Annals and Magazine of Natural History, including*

- Zoology, Botany, and Geology* 7(5): 56.
- Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1,253–1,256.
- Smith A. 1838. Contributions to the natural history of Southern Africa. Article VIII. *Magazine of Natural History* 2(14): 92–94.
- Spawls S, Howell K, Drewes R, Ashe J. 2018. *A Field Guide to the Reptiles of East Africa*. 2nd Edition. Bloomsbury Natural History, London, United Kingdom. 624 p.
- Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758–771.
- Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2,688–2,690.
- Stanley EL, Ceriaco, LMP, Bandeira S, Valerio H, Bates MF, Branch WR. 2016. A review of *Cordylus machadoi* (Squamata: Cordylidae) in southwestern Angola, with the description of a new species from the Pro-Namib desert. *Zootaxa* 4061(3): 201–226.
- Tolley K, Weeber J, Maritz B, Verburgt L, Bates M, Conradie W, Hofmeyr R, Turner A, Da Silva J, Alexander G. 2019. No safe haven: protection levels show imperiled South African reptiles not sufficiently safe-guarded despite low average extinction risk. *Biological Conservation* 233: 61–72.



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The herpetofauna of Bicular National Park and surroundings, southwestern Angola: a preliminary checklist

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Abstract.—Bicular National Park (BNP) is a protected area in southwestern Angola where biodiversity has been poorly studied. BNP is located on the Angolan plateau on Kalahari sands, in a transition zone between the Angolan Miombo Woodland and the Zambezian Baikiaea Woodland ecoregions. Herpetological surveys were conducted in BNP and surrounding areas, through visual encounter surveys, trapping, and opportunistic collecting of specimens from 2015 to 2018. The regional herpetofauna is described here based on these surveys, literature records, and additional unpublished records. In total, 16 amphibian, 15 lizard, 18 snake, two testudine, and one crocodilian species were observed from the recent surveys, and in combination with historical records the species counts are 21, 36, 32, four, and one species for these herpetofauna groups, respectively. Important observations include the first record of *Xenocalamus bicolor bicolor* (Günther, 1868), the second records of *Sclerophrys poweri* (Hewitt, 1935) and of *Amblyodipsas ventrimaculata* (Roux, 1907), and the fourth record of *Monopeltis infuscata* (Broadley, 1997) for Angola. Additionally, the type locality of *Hyperolius benguellensis* (Bocage, 1893) is discussed. A part of the material could not be confidently identified to species level, reflecting the taxonomic uncertainty associated with the Angolan herpetofauna. Fossorial herpetofauna was well represented, reflecting adaptation to sandy soils, the dominant substrate in the area. The likely presence of endemic and poorly known species in BNP reinforces the importance of the park for the conservation of Angolan biodiversity. Further surveys are necessary for a more comprehensive understanding of the park's fauna and biogeographic affinities, and to improve conservation planning.

Keywords. Amphibians, reptiles, fossorial, biodiversity surveys, protected areas, Kalahari sands, Huíla Province

Resumo.—O Parque Nacional do Bicular (BNP) é uma área protegida no sudoeste de Angola cuja biodiversidade se encontra pouco estudada. Localiza-se no planalto de Angola em areias do Calaári, numa zona de transição entre as ecorregiões de Mata de Miombo Angolana e Mata de Baikiaea Zambeziana. Neste trabalho foram realizados levantamentos de herpetofauna no BNP e arredores, através de levantamentos de encontro visual, armadilhagem e recolha oportunística de espécimes entre 2015 e 2018. Aqui é apresentada uma descrição da herpetofauna da região baseada nestes levantamentos, em registos bibliográficos, e outros registos não publicados. Os dados recentes resultaram num total de 16 espécies de anfíbios, 15 espécies de lagartos, 18 espécies de cobras, duas espécies de quelónios, e uma espécie de crocodilo. A combinação destes dados com registos históricos resulta num total de 21, 36, 32, quatro, e uma espécies destes grupos herpetológicos, respectivamente. Entre os resultados mais importantes estão o primeiro registo de *Xenocalamus bicolor bicolor* Günther, 1868, o segundo registo de *Sclerophrys poweri* (Hewitt, 1935) e de *Amblyodipsas ventrimaculata* (Roux, 1907), e o quarto registo de *Monopeltis infuscata* Broadley, 1997 para Angola. A localidade-tipo de *Hyperolius benguellensis* (Bocage, 1893) é também discutida. Uma parte do material não pôde ser identificado com certeza ao nível da espécie, uma consequência da incerteza taxonómica associada à herpetologia angolana. A herpetofauna fossorial está bem representada, reflectindo uma adaptação a solos arenosos, o substrato dominante na área. A presença provável de espécies endémicas e pouco conhecidas no BNP reforça a importância do parque para a conservação da biodiversidade de Angola. Mais levantamentos contribuirão para um melhor conhecimento da fauna do parque e das suas afinidades biogeográficas e para um melhor planeamento de estratégias de conservação.

Palavras-chave. Anfíbios, répteis, fossorial, levantamentos de biodiversidade, áreas protegidas, areias do Calaári, província da Huíla

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Introduction

Angola's biodiversity is poorly understood, especially when compared to other countries in southern Africa (Huntley et al. 2019b). A recent synthesis of the country's biodiversity (Huntley et al. 2019b) provides updated checklists for several taxonomic groups, including amphibians (Baptista et al. 2019) and reptiles (Branch et al. 2019c), as does the historical atlas by Marques et al. (2018), but all these lists highlight the understudied status of the herpetofauna in Angola. Southwestern Angola is one of the better-studied regions for vertebrates in the country (Crawford-Cabral and Mesquitela 1989), and this is reflected in herpetofauna, from which several type descriptions originated. Despite many historical herpetological surveys in the area (Bocage 1895; Schmidt 1933, 1936; Monard 1937a,b; Mertens 1938; Bogert 1940; Laurent 1964; Gans 1976; Poynton and Haacke 1993), contemporary studies continue to yield important discoveries of new species (Haacke 2008; Conradie et al. 2012, 2013; Stanley et al. 2016; Ceriaco et al. 2018b; Branch et al. 2019b), new species records for the country (Huntley 2009; Ceriaco et al. 2016; Branch et al., unpub. data), and rediscoveries of species not observed for several decades (Baptista et al. 2018; Branch et al. 2019a; Vaz Pinto et al. 2019).

The herpetofauna remains undersampled or unsurveyed in vast areas of southwestern Angola, including Bicular National Park (BNP). Wulf Haacke conducted two significant herpetological surveys in this region in 1971 and 1974, collecting over 2,000 specimens (Branch et al. 2019c). Although BNP was not specifically sampled by Haacke, similar habitats near the park were included in the surveys. Poynton and Haacke (1993) described the amphibian collection resulting from these surveys, but information on the new and rare reptiles deposited in the Ditsong National Museum of Natural History (former Transvaal Museum) was never formally published (Branch et al. 2019c). The Angolan civil war prevented research in the country for 35 years (1974–2009) between Haacke's expeditions and the first post-war biodiversity surveys in southwestern Angola (Huntley 2009). The first herpetological surveys inside BNP were part of this effort, during a brief visit by Alan Channing. Only two adult *Hyperolius benguellensis* and several species of tadpoles were collected (Channing et al. 2013; A. Channing, pers. comm.), with the material deposited in Berlin Zoological Museum, and other

observations remained unpublished. More recent surveys include two short visits to BNP in 2017 and 2018 by Butler et al. (2019).

To document the herpetofauna of BNP, we conducted surveys in and around the park from 2015 to 2018 as part of the Southern African Science Service Centre for Climate Change and Adaptive Land Management (SASSCAL) project, which established an observatory in BNP. This is part of a network of five other Angolan observatories, and a total of 47 observatories throughout Southern Africa (Jürgens et al. 2018). Results of herpetological surveys in Tundavala observatory, southwestern Angola, were published recently (Baptista et al. 2018). This paper draws upon records and photographs of BNP herpetofauna partially available since 2017 through SASSCAL's on-line platform (SASSCAL ObservationNet 2018), and is their first formal publication. It presents the results of the 2015–2018 SASSCAL surveys, and it includes collated data from recent and historical collections and observations made by others in and around BNP to provide a more comprehensive account.

Materials and Methods

Study Area

Bicular National Park (BNP) in Huíla Province, southwestern Angola, was declared as a Partial Game Reserve in 1957 to protect populations of big game before being upgraded to National Park status in 1964 (Teixeira 1968; Huntley et al. 2019a). The park was originally 790,000 ha in size (Huntley et al. 2019a), with boundaries described in *Diploma Legislativo* nº 3527 of 26 December 1964 (Teixeira 1968; Simões 1971). In 1972, a governmental decree in *Portaria* 384/72 deproclaimed areas of northern BNP for the expansion of the “Capelongo colonial settlement” (“Colonato de Capelongo”) [L. Veríssimo, pers. comm.], defined the current boundaries of BNP, and decreased the park's area to 675,000 ha (Mendelsohn and Mendelsohn 2018; Huntley et al. 2019a).

BNP lies at ca. 1,200–1,400 m above sea level (asl), between the Cunene and Caculuar rivers on wind-blown Kalahari sands (Mendelsohn and Mendelsohn 2018). This is the most extensive contiguous body of sand in the world (Leistner 1967) and extends from the southern African plateau to the Congo basin. The park

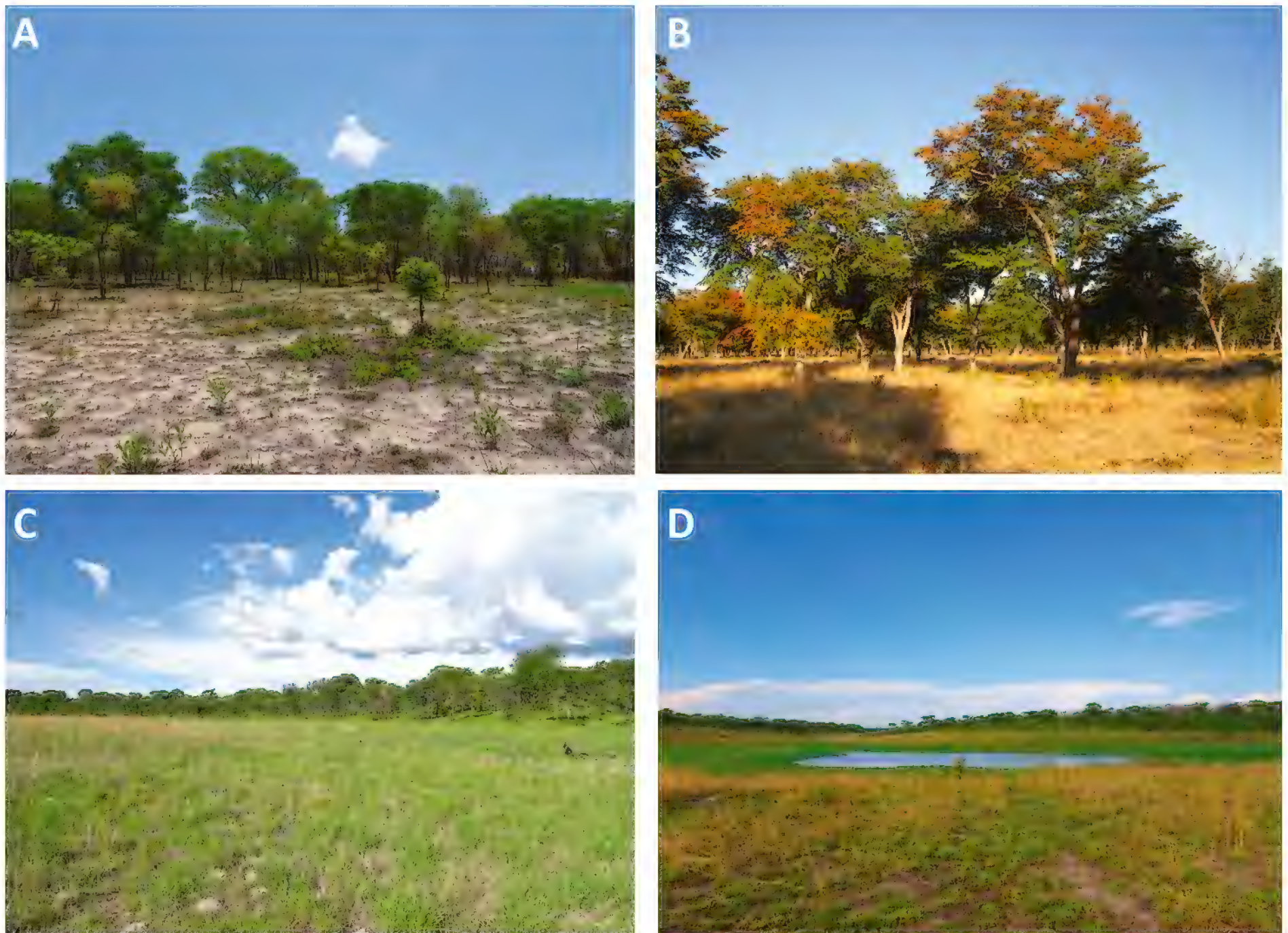


Fig. 1. Habitat types in the study area. (A) Angolan Miombo woodland on Kalahari sands, BNP; (B) *Burkea/Baikiaea* woodlands, Carmira Farm; (C) dry grassy wetland (“mulola”) flanked by miombo woodlands (“tunda”), BNP; (D) temporary pan in a “mulola,” BNP.

is part of the “Lower Cunene” mesological unit (Diniz 2006; Huntley 2019), and is located in a transition zone from moist to dry savannas. It comprises both the Angolan Miombo Woodland and Zambezian *Baikiaea* Woodland ecoregions, as defined by Burgess et al. (2004), which are equivalent to the *Brachystegia* and South-west Arid biomes, respectively, as characterized by Huntley (1974). The northern extent is dominated by miombo woodlands, consisting mostly of *Brachystegia spiciformis* and *Julbernardia paniculata*, while the south is covered predominantly by savannas dominated by *Burkea africana* (Teixeira 1968) [Fig. 1], and Angolan Mopane woodlands are present to the south of the park (Huntley 2019). In addition to woodlands, thickets, and scrublands of varied composition, open drainage lines hosting grasslands with geoxyllic suffrutex shrublands are common throughout; see Teixeira (1968), Barbosa (1970), and Chisingui et al. (2018) for further details.

Elliptical in shape, the park is ca. 80 km in diameter from north to south and ca. 110 km from east to west (Fig. 2). The climate is seasonal, with precipitation falling mainly from October to April, and nocturnal frost occurring frequently in the dry season, especially in June and July. Meteorological data for BNP observatory are available from 2015 onwards (SASSCAL WeatherNet

2019). The average annual rainfall varies from ca. 900–950 mm in the northern border and 650–700 mm in the southern border, and average annual temperatures vary between ca. 19–20 °C in the north and 22–23 °C in the south, with values for north and south taken from Quipungo and Mulondo, respectively, in Mendelsohn and Mendelsohn (2018).

Topographically BNP is generally flat, interrupted by relatively parallel drainage lines mostly flowing west to east (Fig. 2). The park is divided through the center by a larger depression, called Bicular, which flows in the north-south direction and forms part of the Cunene River catchment (Teixeira 1968). Natural grasslands with geoxyllic suffrutex shrublands occur in drainage lines that are seasonally filled with water (locally called “mulolas”) [Fig. 1D] where cold air accumulates at night during the dry season. Many of the existing permanent water bodies in the park are artificial excavations made to attract game for observation purposes (Simões 1971). The park’s elevated regions (locally called “tundas”) are covered by woodlands, and are only 30–50 m higher in altitude than the valleys with grasslands. BNP’s soils are mostly sandy (Missão de Pedologia de Angola 1959; Teixeira 1968), and arenosols as defined by Jones et al. (2013), with rare rocky outcrops. The landscape surrounding BNP

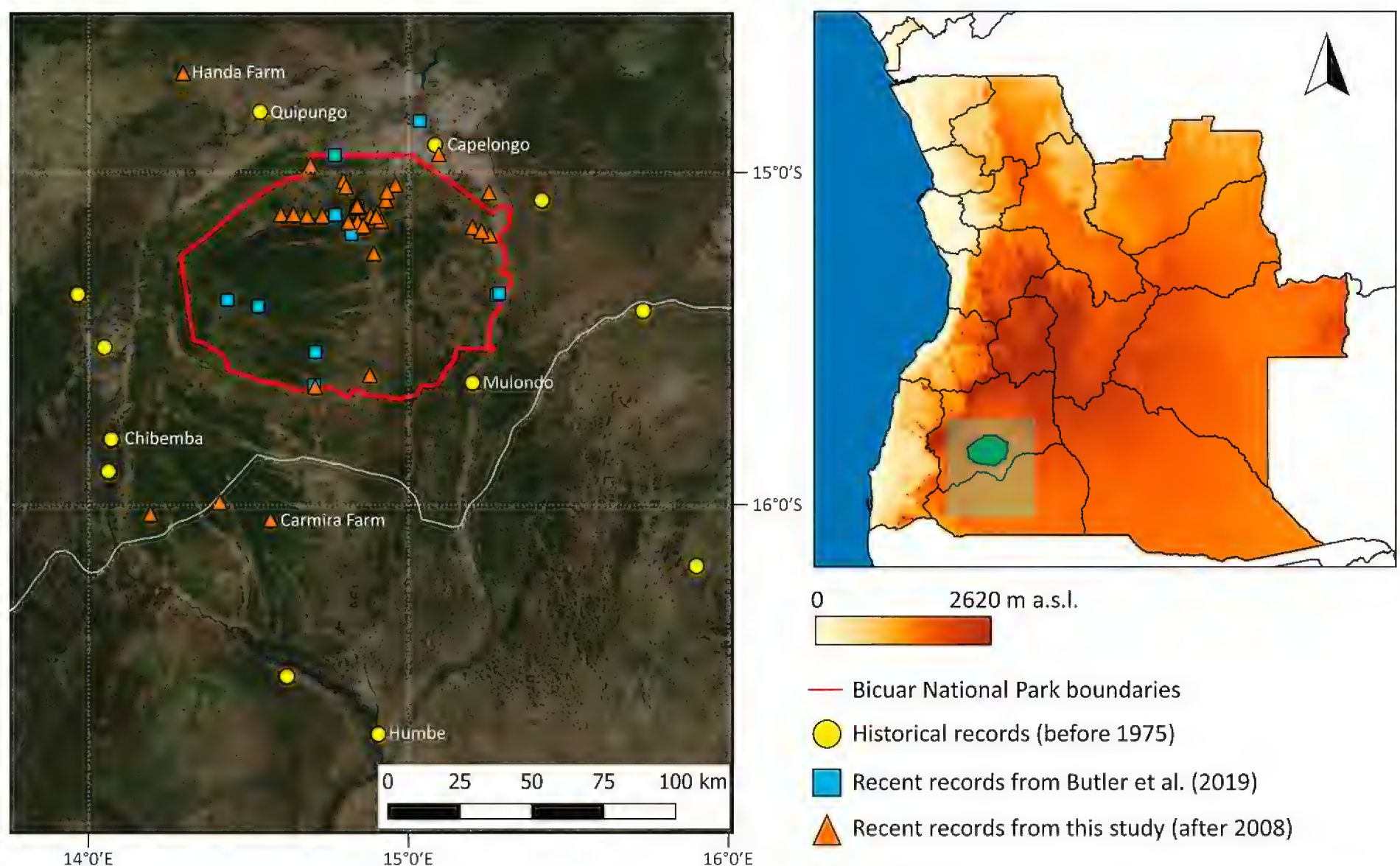


Fig. 2. Left: Bicular National Park (BNP) and surveyed sites from historical and recent records. Right: Angola with provincial boundaries and location of BNP. Source of satellite image: Maxar (<https://www.digitalglobe.com/>).

is similar (Barbosa 1970; Mendelsohn and Mendelsohn 2018) such that records from localities surrounding the park are also reported in this work.

Survey Methods and Data Sources

For this study, surveys of herpetofauna were performed in BNP from October 2015 to April 2018. These included opportunistic collections during occasional visits to the park, and two series of focused surveys, from 2–10 December 2016 (during the rains) and 3–7 November 2017 (at the onset of the rains). For both surveys, diurnal and nocturnal Visual Encounter Surveys (VES) were performed, as well as dipnetting in water bodies by scooping the bottom, using nets of varied shape and mesh size. Trap arrays consisting of drift fences with pitfall and funnel traps were set during the 2016 survey at three sites within the following habitats: miombo woodlands not burnt for more than one year (site T1, see Appendix 1 for coordinates and duration), miombo woodlands not burnt for more than five years (T2, Appendix 1), and grassland along a drainage line (T3, Appendix 1). Each trap array consisted of one plastic drift fence (15 m long and 50 cm high) with two pitfall traps, one at each end of the drift fence, and six funnel traps placed on adjacent sides of the fence. Collecting sites in BNP and the surroundings included the Main Camp and permanent water bodies (waterhole near the Main Camp, Lagoa da Matamba, Lagoa do Djimbi, and Lagoa Nougafafa, among others). These sites are mapped in Fig. 2, and geographic

coordinates are provided in Appendix 1. Surveyed habitat types included miombo and *Burkea/Baikiaea* woodlands, ponds, and excavations in drainage lines where water is permanently provided by water pumps. Large portions of the surveyed areas in the north were burnt two to four weeks prior to being surveyed.

Additional observations include records from Channing's 2009 visit (A. Channing, pers. comm.), as well as opportunistic records from other researchers working in the region (Manfred Finckh and Francisco Maiato, who provided photographs), and from two farms located near the park. Carmira Farm, located ca. 40 km northeast of Cahama and ca. 46 km south of BNP (site CF), has staff with a personal interest in collecting reptiles, as they have created a collection (Fig. 3) comprising snakes, amphisbaenians, and writhing skinks. Handa Farm, 30 km northwest of Quipungo and 48 km northwest of BNP (site HF), maintains a collection of photographic records of its fauna, including reptiles. All recent records in these sources were considered for this study, and all except Channing's 2009 data (A. Channing, pers. comm.) were verified by the authors. Butler et al. (2019) addressed BNP herpetofauna, and these results have also been incorporated into this work.

Collected specimens were photographed and euthanized by either submersion (in the case of frogs) or injection into the intracoelomic cavity (for reptiles) of a solution of tricaine methanesulfonate (MS222) [Conroy et al. 2009]. Tissue (liver or muscle) was preserved in 99.5% ethanol for genetic analysis. Specimens were fixed



Fig. 3. Carmira Farm private collection.

with formalin, then transferred to water (to remove the formalin), before finally being transferred to 70% ethanol for long-term storage. All photographs were taken by the first author (N. Baptista), except when noted. Specimens are held in the herpetological collection of Instituto Superior de Ciências da Educação da Huíla (ISCED-Huíla), Lubango, Angola. Additional specimens that were not collected for this study are deposited in Carmira Farm's private collection (Fig. 3).

Field guides (Branch 1998; Schiøtz 1999; Channing 2001; Marais 2004; du Preez and Carruthers 2009; Channing et al. 2012; Channing and Rödel 2019) were used for species identification, and additional taxon-specific literature was consulted when necessary. Nomenclature followed online databases: Amphibian Species of the World (Frost 2019) for amphibians, and The Reptile Database (Uetz et al. 2019) for reptiles, and was updated when appropriate. Only materials from the ISCED-Huíla collection and Carmira and Handa Farms were examined for this study. Taxonomic identification of historical records (published in the literature, and unpublished records from Haacke's surveys) and acoustic survey records may require verification.

Localities for historical data compiled for the BNP region (yellow dots in Fig. 2) were selected based on distances to the park (i.e., those within a 100 km radius of the park's boundaries) and physiographic similarity (Huntley 2019). The only exception to this is Humbe, which is located 110 km south of the park, but was also included in this account since it is an important historical collection site and is the type locality of several reptile species. Selected historical

localities were: Cahama and surroundings, Capelongo (= Kapelongo, = Folgares), Catequero and surroundings, Chibemba and surroundings, Dongue and surroundings, Gambos (= Chibemba, see discussion in Branch et al. [2019a]), Humbe, Humbi (= Humbe), Humbia (= Humbe), Kandingu (= Kului River), Kangela (= Kului River), Kului River, Mulondo, Mupa, Osi (= Osse), Osse, Quipungo, and Viriambundo. Records from these localities were compiled from published literature such as Bocage (1895), Monard (1937a,b), Schmidt (1933), Bogert (1940), Laurent (1964), Gans (1976), Poynton and Haacke (1993), and from unpublished information in the Ditsong (=Transvaal) National Museum of Natural History's database (Haacke, TM).

Collected data were grouped into three classes (Fig. 2): i) historical records collected before 1975, which include records published in historical literature and unpublished records from TM; ii) recent records from Butler et al. (2019); and iii) recent records from this study, which refer to either data collected during field surveys from 2009 onwards performed within the scope of the SASSCAL project, A. Channing's personal records, opportunistic photographic records collected by other researchers in the region, and records from Carmira Farm and Handa Farm.

Results

The combined records (published and unpublished, recent and historical) of herpetofauna biodiversity from the BNP region comprises a total of 94 taxa. This includes 21 amphibian taxa (Table 1) and 73 reptilian taxa (36 lizards,

Table 1. Amphibians recorded from inside and the surroundings of Bicular National Park, Angola, based on historical and recent records. Type of record: C = advertisement call; L = literature; O = observation; P = photograph; RR = new record for the region; V = voucher. Period of record: A = after 2008; B = before 1975. Taxonomy has been updated over the years, therefore original species citations may occur under different names.

	Species	Type of record	Records in the region of BNP Locality (Reference)	Inside BNP?	Period of record
Arthroleptidae					
1.	<i>Leptopelis bocagii</i> (Günther, 1865)	RR, V	BNP (this study)	Y	A
Bufonidae					
2.	<i>Mertensophryne</i> aff. <i>mocquardi</i> (Angel, 1924)	L	Mulondo (Monard 1937a)		B
3.	<i>Sclerophrys poweri</i> (Hewitt, 1935)	RR, V	BNP (this study)	Y	A
4.	<i>Sclerophrys pusilla</i> (Mertens, 1937)	L, V	Capelongo (Butler et al. 2019); BNP (this study)	Y	A
5.	<i>Sclerophrys regularis</i> (Reuss, 1833)	L	Humbe, Mulondo, Mupa, Osi (= Osse) (Monard 1937a)		B
Hemisotidae					
6.	<i>Hemisus</i> cf. <i>guineensis</i> Cope, 1865	RR, V	BNP (this study)	Y	A
Hyperoliidae					
7.	<i>Hyperolius angolensis</i> Steindachner, 1867 complex	L, V	Capelongo, Osi (= Osse) (Monard 1937a); BNP (Butler et al. 2019; this study)	Y	A, B
8.	<i>Hyperolius benguellensis</i> (Bocage, 1893) complex	V	BNP (Channing et al. 2013); BNP (Butler et al. 2019; this study)	Y	A
9.	<i>Kassina senegalensis</i> (Duméril and Bibron 1841)	L, V	Mulondo (Monard 1937a); BNP (this study)	Y	A, B
Microhylidae					
10.	<i>Phrynomantis bifasciatus</i> (Smith, 1847)	C, L	Mulondo (Monard 1937a); BNP (this study)	Y	A, B
Phrynobatrachidae					
11.	<i>Phrynobatrachus mababiensis</i> FitzSimons, 1932	C, L, V	BNP (Butler et al. 2019; this study)	Y	A
12.	<i>Phrynobatrachus natalensis</i> (Smith, 1849)	C, L	Kangela (Monard 1937a); BNP (this study)	Y	A, B
Pipidae					
13.	<i>Xenopus petersii</i> Bocage, 1895	L, V	Kandingu, Osi (= Osse) (Monard 1937a); BNP (Butler et al. 2019; this study)	Y	A, B
Ptychadenidae					
14.	<i>Hildebrandtia</i> cf. <i>ornata</i> (Peters, 1878)	L, P	Mulondo, Osi (= Osse) (Monard 1937a); Dongue (Poynton and Haacke 1993); between Chibemba and Cahama (this study)		A, B
15.	<i>Ptychadena ansorgii</i> (Boulenger, 1905)	L	Kandingu (Monard 1937a)		B
16.	<i>Ptychadena oxyrhynchus</i> (Smith, 1849)	L, V	Osi (= Osse) (Monard 1937a); BNP (Butler et al. 2019; this study)	Y	A, B
17.	<i>Ptychadena porosissima</i> (Steindachner, 1867)	C, RR	BNP (this study)	Y	A
Pyxicephalidae					
18.	<i>Pyxicephalus adspersus</i> Tschudi, 1838	L, O	Humbe (Bocage 1895); Carmira Farm (this study)		A, B

Table 1 (continued). Amphibians recorded from inside and the surroundings of Bicular National Park, Angola, based on historical and recent records. Type of record: C = advertisement call; L = literature; O = observation; P = photograph; RR = new record for the region; V = voucher. Period of record: A = after 2008; B = before 1975. Taxonomy has been updated over the years, therefore original species citations may occur under different names.

	Species	Type of record	Records in the region of BNP Locality (Reference)	Inside BNP?	Period of record
19.	<i>Tomopterna ahli</i> (Deckert, 1938)	L	BNP (Butler et al. 2019; Channing and Becker 2019)	Y	A
20.	<i>Tomopterna</i> cf. <i>cryptotis</i> (Boulenger, 1907)	C, L, V	Catequero (Boulenger 1907); Calequero (= Catequero), 2 km NW of (Poynton and Haacke 1993); BNP (this study)	Y	A, B
21.	<i>Tomopterna tuberculosa</i> (Boulenger, 1882)	L	BNP (Butler et al. 2019)	Y	A

32 snakes, four testudines, and one crocodilian, Table 2). Many of Haacke’s data represent new records for the region (see Table 2), highlighting the relevance of his collections. The recent records resulting from this study recorded a total of 53 taxa; 16 amphibian taxa distributed across nine families and 13 genera (Table 1), and 36 reptilian taxa (15 lizards, 18 snakes, two testudines, and one crocodilian) distributed among 17 families and 35 genera (Table 2). This study revealed the first record of *Xenocalamus bicolor bicolor*, the second records of *Sclerophrys poweri* and of *Amblyodipsas ventrimaculata* (together with the record of Butler et al. [2019]), and the fourth record of *Monopeltis infuscata* in Angola. In total, 89 specimens were collected and deposited in the herpetological collection of ISCED-Huíla.

Comments on the recent records from this study are provided in the species accounts below, which are arranged alphabetically by class, family, genus, and species, with notes on taxonomy and the relevance of the discoveries. For each species, the material used is described as the type of record, the field number of the collected specimen when applicable, and a code in brackets which represents the site where the specimen was recorded (see Appendix 1). Some records could not be assigned to a species with certainty because no voucher specimens were available for verification (e.g., *Hildebrandtia*, *Afrotyphlops*), or due to taxonomical uncertainty in the group to which they belong (e.g., *Hyperolius*, *Hemisus*, *Panaspis*). In these cases, the nomenclature followed Sigovini et al. (2016). Collected tadpoles were not identified and are therefore not listed as materials below, and their future identification awaits the results of DNA sequencing. The only exceptions to this exclusion are the tadpoles assigned to *Kassina*.

Species Accounts

Amphibia

Arthroleptidae

Leptopelis bocagii (Günther, 1865)
Bocage's Burrowing Treefrog (Fig. 4A–B)

Material: NB520 (30); NB546 (28); NB547 (29); NB710 (27); NB765 (27); NB766 (27).

Comment: Reported in several localities in Angola (Ceríaco et al. 2018c; Marques et al. 2018), including recent records from Huíla Province (Baptista et al. 2018). It is considered a complex of cryptic species (Schjötz 1999), and the Angolan material assigned to *Hylambates angolensis* Bocage, 1893, currently in the synonymy of *L. bocagii*, requires further study (Perret 1976). Dorsal coloration of individuals in BNP varied from completely



Fig. 4. *Leptopelis bocagii*, BNP. (A) female with plain back; (B) male with blotch in back.

plain to black with a horseshoe shaped blotch with white dots in the back (Fig. 4A–B).

Bufonidae

Sclerophrys poweri (Hewitt, 1935)

Western Olive Toad (Fig. 5)

Material: NB512 (36); NB756 (25); NB764 (26); NB767 (26); NB768 (26); NB769 (26).

Comment: Also occurring in northern Namibia and Botswana (Channing 2001), this is the second confirmed record of the species for Angola, after a previous record near Calai (Conradie et al. 2016). Some early records of *Bufo regularis sensu latu* may also refer to *S. poweri* (Ruas 1996). *Bufo regularis humbensis* Monard, 1937 was originally described from Mulondo, close to BNP. It is currently placed under the synonymy of *S. garmani* (Tandy and Keith 1972), which in Angola refers to *S. poweri*, and this synonymy should be reviewed. *Sclerophrys poweri* is very abundant in BNP and was found hundreds of meters away from water on moist nights. Breeding was observed in permanent pans, with males calling in choruses at night and eggs typically laid in gelatinous single strings.

Sclerophrys pusilla (Mertens, 1937)

Southern Flat-backed Toad (Fig. 6)

Material: NB056 (31); NB763 (26).

Comment: Taxonomy and identification of bufonids in Angola remains problematic (Baptista et al. 2019). *Sclerophrys pusilla* represents populations previously assigned to *S. maculata* in eastern and southern Africa, including Angola (Poynton et al. 2016). This species is known from several localities in the country (Poynton and Haacke 1993; Ruas 1996, 2002; Conradie et al. 2016; Ceriaco et al. 2018c; Marques et al. 2018). It was heard calling in BNP on 24 January 2009, but not collected (A. Channing, pers. comm.), and was recently recorded from Capelongo, near BNP (Butler et al. 2019). It was also found around the Main Camp facilities and in a permanent water body.

Hemisotidae

Hemisus cf. *guineensis* Cope, 1865

Guinea Snout-burrower (Fig. 7A–C)

Material: NB511 (15); NB550 (T2); NB551 (T2).

Comment: Tadpoles of this species were collected in BNP during the 2009 survey (A. Channing, pers. comm.). The taxonomy of *Hemisus guineensis* and *H. marmoratus* is not fully resolved. Angolan specimens of both species have been treated as a single taxon in the past (*Hemisus guineensis microps* Laurent, 1972) and records of the genus are scattered throughout the country (Ruas 1996; Marques et al. 2018). Recent records of *Hemisus* from Cangandala have been considered as *H. guineensis* (Ceriaco et al. 2018c; Vaz Pinto and Baptista,

unpub. data). Coloration of adult frogs from BNP ranges from finely spotted forming lines, and mottled forming continuous blotches to almost plain with very small spots (see Fig. 7A–C). A similar pattern of variation in coloration was reported by W. Conradie (pers. comm.) in southeastern Angola, with all forms genetically confirmed as being *H. cf. guineensis*. Based on this, and the proximity to a previous record from the Cubango basin (Monard 1937a), BNP specimens likely belong to the same taxon.

Hyperoliidae

Hyperolius angolensis Steindachner, 1867 complex

Angola Reed Frog

Material: NB523 (41); NB524 (41); NB538 (27); NB539 (27); NB540 (27); NB541 (27).

Comment: This species was heard calling in BNP (site 31) on 24 January 2009, but no material was collected (A. Channing, pers. comm.). It was recently recorded in BNP by Butler et al. (2019), who provided photos of coloration variations. This is another unresolved complex of reed frogs in Africa (see Schiøtz 1999), with a number of names available for Angolan populations (Baptista et al. 2019, as *H. parallelus*). In Angola, this complex is recorded throughout the country (Monard 1937a; Laurent 1950, 1954, 1964; Poynton and Haacke 1993; Conradie et al. 2016; Baptista et al. 2018; Ceriaco et al. 2018c; Marques et al. 2018), with consistent regional color patterns. Poynton and Haacke (1993) referred to previous records in Huíla Province as *Hyperolius marmoratus huillensis*.

Hyperolius benguellensis (Bocage, 1893) complex

Benguela Long Reed Frog (Fig. 8A–B)

Material: NB510 (36); NB526 (41); NB542 (27); NB543 (27); NB544 (27).

ZMB 77273; ZMB 77274 (collected by Alan Channing, not analyzed).

Comment: *Hyperolius benguellensis* belongs to the challenging *Hyperolius nasutus* complex, with a problematic taxonomy throughout Africa (Schiøtz 1999; Amiet 2005; Marques et al. 2018). Channing et al. (2013) proposed a rearrangement for this group based on morphology, genetics, and advertisement calls, resulting in four species occurring in Angola: *H. nasutus*, *H. dartevellei*, *H. adpersus*, and *H. benguellensis*. Of these, three were originally described from Angola: *Hyperolius nasutus* Günther, 1865, type locality: Duque de Bragança (= Calandula); *Hyperolius adpersus* Peters, 1877, type locality: Chinchoxo, in Cabinda; and *Hyperolius benguellensis* (Bocage, 1893) type locality: "Cahata" that has been incorrectly assigned to Caota (e.g., Channing 2001; Marques et al. 2018; Frost 2019). In fact, Cahata is located 5 km east of Balombo Municipality and was a known collecting site for the famous naturalist José de Anchieta in the nineteenth century (Bocage 1895).



Fig. 5. *Sclerophrys poweri*, BNP



Fig. 6. *Sclerophrys pusilla*, BNP



Fig. 7. *Hemisus* cf. *guineensis*, BNP. (A–C) Three variations in coloration.

Although the region is still located in Benguela Province, it lies on the plateau at 1,230 m asl, more than 400 km east of the coastal town of Benguela and closer to the town of Huambo, while Caota is found on the outskirts of Benguela at 20 m asl. The name “*benguellensis*” is therefore misleading, and is probably the reason why Cahata was confused with Caota, but the latter is a beach site in the semi-arid Angolan southwest, and an unlikely habitat for a reed frog. We therefore re-establish the type locality of *H. benguellensis* to Cahata, near Balombo. The assignment of erroneous names to Angolan localities has been detected in other cases (Branch et al. 2017, 2018; Vaz Pinto et al. 2019), thus special attention must be given to this when consulting historical and recent literature. Channing et al. (2013) assigned one specimen from BNP to *H. benguellensis*, and we assign these specimens to this name, with the altitude similar to the true type locality providing further support. Butler et al. (2019) collected one specimen from BNP, recording it as *H. cf. nasutus*, and in this study we tentatively regard this record as the same as ours. Both species, *H. benguellensis* and *H. nasutus*, are sympatric in Cangandala National Park (Vaz Pinto, unpub. data). Specimens from this group have been also found in western Zambia (Bittencourt-Silva 2019) and assigned to *H. dartevillei* and *H. nasicus*. Similar to *H. angolensis* complex, a more complete understanding of this complex in Angola requires countrywide surveys and an integrated analysis of molecular, morphological, and advertisement call data.

Kassina senegalensis (Duméril and Bibron, 1841)

Bubbling Kassina

Material: NB525 (T1); NB536 (tadpoles) (7); NB545 (T3); NB552 (T2); NB761 (26).

Comment: Widespread throughout Africa and in Angola (Baptista et al. 2018; Marques et al. 2018). This species has been previously recorded in BNP as tadpoles (A. Channing, pers. comm.). Although subspecies have been proposed based on color patterns (Laurent 1957), more comprehensive studies are required to resolve this species' taxonomy.

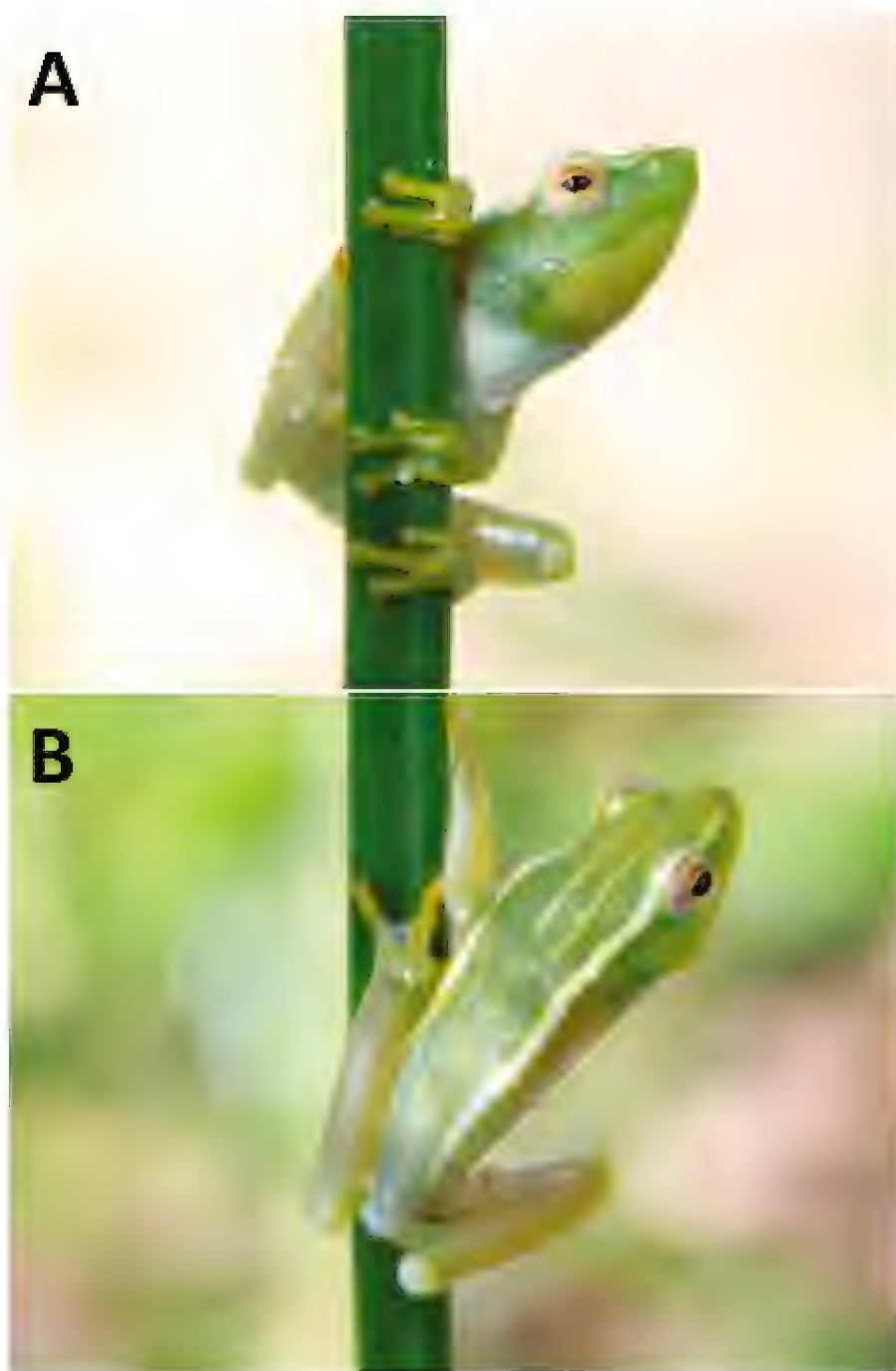


Fig. 8. *Hyperolius benguellensis* complex, BNP. (A) Ventral view; (B) dorsal view.

Microhylidae

Phrynomantis bifasciatus (Smith, 1847)

Banded Rubber Frog

Comment: This species was heard calling in BNP (site 31) on 24 January 2009, but no material was collected (A. Channing, pers. comm.). In Angola, it is known from Mulondo (Monard 1937a), which is located near the boundary of BNP, in addition to Quissange and Benguela (Bocage 1895), and Chingo (Ferreira 1904).

Phrynobatrachidae

Phrynobatrachus mababiensis FitzSimons, 1932

Mababe Puddle Frog

Comment: This species was heard calling in BNP (site 31) on 24 January 2009, and a single tadpole (A. Channing, pers. comm.) and adults (Butler et al. 2019) have been collected in BNP. In Angola, it is recorded from Lagoa Nuntechite (Poynton and Haacke 1993), Cubango, Cuito and Cuando rivers basins (Conradie et al. 2016), and Tundavala (Baptista et al. 2018). This species belongs to the *P. cryptotis* group (Marques et al. 2018) and is in need of further taxonomic studies all over Africa (Zimkus et al. 2010).

Phrynobatrachus natalensis (Smith, 1849)

Snoring Puddle Frog

Comment: This species was heard, but not recorded, calling in BNP (site 31) on 24 January 2009; no material was collected (A. Channing, pers. comm.). It is widespread in Angola (Ruas 1996, 2002; Marques et al. 2018) and includes several cryptic taxa (Zimkus et al. 2010) that are presently being investigated. Additional specimens collected from BNP will be crucial for species confirmation.

Pipidae

Xenopus petersii Bocage, 1895

Peters' Clawed Frog

Material: NB757 (27); NB758 (27); NB759 (27).

Comment: This species is widespread in Angola (Monard 1937a as *X. laevis*; Ruas 1996, 2002; Baptista et al. 2018; Ceriaco et al. 2018c; Marques et al. 2018). It has been previously recorded in BNP from both tadpoles (A. Channing, pers. comm.) and adults (Butler et al. 2019). Furman et al. (2015) consider that in Angola, *X. petersii* is widespread and *X. poweri* is restricted to southeastern Angola, but few samples from Angola were used in their analysis and this genus is worthy of a more comprehensive assessment in the country. Hamerkop (*Scopus umbretta*) and Lilac-breasted Roller (*Coracias caudatus*) were seen preying upon this species in BNP.

Ptychadenidae

Hildebrandtia cf. *ornata* (Peters, 1878)

Ornate Frog (Fig. 9)

Material: Photographic record (F. Maiato, on wetland between Chibemba and Cahama, approximate coordinates same as site 42).

Comment: Two species of *Hildebrandtia* exist in Angola: *H. ornata* and *H. ornatissima* (Marques et al. 2018; Baptista et al. 2019). *Hildebrandtia ornata* is limited to the southwest, and *H. ornatissima* extends northwards to the central plateau (Ruas 1996; Marques et al. 2018). Boulenger (1919) provides morphological distinction between *H. ornatissima* (Bocage, 1897), endemic to Angola, and *H. ornata* (Peters, 1878) (= *R. ruddi*), originally described from Kenya but with a wide distribution in Africa. Perret (1976) considered *H. ornata* and *H. ornatissima* as two valid species based on morphology, but Poynton and Haacke (1993) contest that, and consider them subspecies. Specimens collected from Dongue, 28 km west of BNP and other localities in southwestern Angola had mixed features from both taxa and were assigned to *H. ornata* (Poynton and Haacke 1993). A single frog was photographed, approximately 55 km southwest of BNP's boundaries, and it is provisionally assigned to *H. ornata* based on the identification of material collected in close proximity (Poynton and Haacke 1993). *Hildebrandtia*



Fig. 9. *Hildebrandtia* cf. *ornata*, between Chibemba and Cahama (Photo by F. Maiato).

specimens have also been recently collected from several localities in Huíla and Malanje provinces (Baptista and Vaz Pinto, unpub. data), and the genus requires a revision in the country.

Ptychadena oxyrhynchus (Smith, 1849)

Sharp-nosed Ridged Frog (Fig. 10A–B)

Material: NB750 (25).

Comment: Species identification for *Ptychadena* in Angola is not resolved (Baptista et al. 2019). A single specimen was found near Lagoa da Matamba, but no advertisement call was heard. It was assigned to *P. oxyrhynchus* based on morphology and coloration (Fig. 10A–B). This species has recently been recorded from BNP (Butler et al. 2019) and is known from several localities in Angola (Marques et al. 2018).

Ptychadena porosissima (Steindachner, 1867)

Grassland Ridged Frog

Comment: This species was heard calling in BNP (site 31) on 24 January 2009, but no material was collected and no call was recorded (A. Channing, pers. comm.). Widespread in sub-Saharan Africa, and recorded in Angola from the west and the northeast (Ruas 1996; Marques et al. 2018). Additional specimens will be important for confirmation of species identification in BNP.

Pyxicephalidae

Pyxicephalus adspersus Tschudi, 1838

African Bullfrog

Material: Interviews.

Comment: Staff from Carmira Farm mentioned that this frog—which is unmistakable by its distinctive size, morphology, and behavior—appears during the first

heavy rains. *Pyxicephalus adspersus* is recorded from southern Angola in Humbe (Bocage 1895), Mupanda (Monard 1937a), and Pereira d'Eça (= Ondjiva) and 23 km NW Pereira d'Eça (Poynton and Haacke 1993, as *P. a. edulis*). Several synonymies exist within *P. adspersus* (Frost 2019). Consensus regarding whether *P. edulis* occurs in the Zambezi Region (previously the Caprivi Strip), has not been reached (Herrmann and Branch 2013), and the Angolan material requires further analysis. Locally called “mafuma,” it is captured for consumption and bushmeat trade. It may also have cultural relevance in Angola, as in some parts of Cunene Province this frog’s mating behavior is said to inspire a traditional fight called “engolo” among the Nkhumbi people (J. Moniz, pers. comm.).

Tomopterna cf. *cryptotis* (Boulenger, 1907)

Cryptic Sand Frog (Fig. 11A–C)

Material: NB751 (25); NB752 (25); NB753 (25); NB754 (25); NB755 (25); NB762 (26).

Comment: The species was found breeding in early December, with males calling in loud choruses on banks of water bodies in BNP. Collected specimens had considerable coloration variation (see Fig. 11A–C). *Tomopterna cryptotis* was originally described from Catequero (ca. 95 km south of BNP), and has been recorded between Calequero (= Catequero) and Cahama (ca. 75 km southwest of BNP) [Poynton and Haacke 1993] among other localities in Angola (Ruas 1996; Marques et al. 2018). This species is morphologically indistinguishable from *T. tandyi* (Channing and Bogart 1996), which was originally described from the Eastern Cape in South Africa and is known from Namibia, Botswana, and South Africa (Channing 2001, du Preez and Carruthers 2009). Although *T. tandyi* is considered as being present in southwestern Angola (Channing 2001; Channing and Rödel 2019), there are no literature records from the country, and its occurrence is presumably based on the morphology of similar frogs in Namibia (A. Channing, pers. comm.). However, *Tomopterna* species are highly cryptic, and difficult to distinguish based on morphology. Due to the fact that the BNP records are near-topotypical, these specimens are assigned to *T. cryptotis*, but the advertisement calls heard in BNP resembled those of *T. tandyi* provided by du Preez and Carruthers (2019). Further integrative revision of these species in Angola is needed to confirm this assignment. Two other species from the same genus have recently been recorded from BNP (Butler et al. 2019): *T. tuberculosa* and *T. damarensis*, which was recently re-assigned to *Tompterna ahli* (Channing and Becker 2019), which means that at least three species may occur sympatrically. Given the highly cryptic morphology of species within this genus (Channing and Rödel 2019), and the continuing descriptions of new species (Wilson and Channing 2019), all of these new records require revision.



Fig. 10. *Ptychadena oxyrhynchus*, BNP. (A) Individual; (B) detail of thighs.

Reptilia
Squamata
Sauria
Agamidae

Acanthocercus sp.

Tree Agama (Fig. 12)

Material: NB709 (31).

Comment: This species was previously assigned to *Acanthocercus atricollis* (Smith, 1849) until Wagner et al. (2018) revived the name *A. cyanocephalus* for western populations, which included Angola. Reported in several publications from Angola (Bocage 1879, 1895; Ferreira 1902; Boulenger 1905; Monard 1931, 1937b; Schmidt 1933; Laurent 1950, 1964; Manaças 1963; Conradie et al. 2016; Ceríaco et al. 2018c; Marques et al. 2018), but material from Huíla Province belongs to a different species, which is in the process of being described (Marques et al. 2018; Butler et al. 2019). Recently recorded in BNP (Butler et al. 2019), abundant at the park's headquarters, and found on large and tall *Burkea africana* trees.

Agama aculeata (Merrem, 1820)

Ground Agama

Material: NB770 (32).

Comment: Ground Agamas occur widely in southern Africa (Bates et al. 2014) and are reported throughout Angola (Marques et al. 2018). The taxonomy of Angolan populations remains unresolved, and a recent phylogeny of *Agama* (Leaché et al. 2014) did not include Angolan



Fig. 11. *Tomopterna cf. cryptotis*, BNP. (A–C) Three variations in coloration.

samples. Recently recorded in BNP (Butler et al. 2019), in this study it was found on the ground in degraded shrubland in the outskirts of BNP.

Amphisbaenidae

Monopeltis cf. anchietae (Bocage, 1873)

Anchieta's Spade-snouted Worm Lizard (Fig. 13)

Material: One photographic record of a freshly killed individual (L. Gata, CF).

Herpetofauna of Bicular National Park, Angola

Table 2. Reptiles recorded from inside and the surroundings of Bicular National Park, Angola, based on historical and recent records. Type of record: CR = new record for the country; H = unpublished record from Ditsong (= Transvaal) Museum's collection; L = literature; O = observation; P = photograph; RR = new record for the region; V = voucher; E = endemic. Period of record: A = after 2008; B = before 1975. Taxonomy has been updated over the years, therefore original species nomenclature may occur under different names.

Taxa		Endemic?	Type of record	Records in the region of BNP Locality (and Reference)	Inside BNP?	Period of record
Sauria						
Agamidae						
1.	<i>Acanthocercus</i> sp.	E	L, V	Mupa (Monard 1937b); BNP (Butler et al. 2019; this study)	Y	A, B
2.	<i>Agama aculeata</i> (Merrem, 1820)		H, L, V	Cahama and Chibemba, 21 km NW of (Haacke, TM); BNP (Butler et al. 2019; this study)	Y	A, B
3.	<i>Agama planiceps shacki</i> Mertens, 1938	E	H, RR	Chibemba, 5 km S; Dongue, 10 km NW of (Haacke, TM)		A, B
Amphisbaenidae						
4.	<i>Monopeltis</i> cf. <i>anchietae</i> (Bocage, 1873)		L, V	Humbe (Bocage 1873); Mupa (Monard 1937b); Carmira Farm (this study)		A, B
5.	<i>Monopeltis infuscata</i> Broadley, 1997		L, V	Humbe (Bocage 1873; Broadley 1997); Carmira Farm (this study)		A, B
6.	<i>Monopeltis perplexus</i> Gans, 1976	E	L	Humbe (Gans 1976)		B
7.	<i>Zygaspis quadrifrons</i> (Peters, 1862)		L, V	BNP (Butler et al. 2019); Carmira Farm (this study)	Y	A, B
Chamaeleonidae						
8.	<i>Chamaeleo dilepis quilensis</i> (Bocage 1886)		H, L, V	Cahama, 23 km SE (Haacke, TM); Kului (Monard 1937b); BNP (Butler et al. 2019; this study)	Y	A, B
Cordylidae						
9.	<i>Cordylus machadoi</i> Laurent, 1964		H, RR	Chibemba, 5 km S; Humbia, 12 km E (Haacke, TM)		B
Gekkonidae						
10.	<i>Chondrodactylus fitzsimonsi</i> (Loveridge, 1947)		H, RR	Viriambundo (Haacke, TM)		B
11.	<i>Chondrodactylus laevigatus</i> (Fischer, 1888)		L, O	Mulondo (Schmidt 1933); Humbe (Monard 1937b); BNP (Butler et al. 2019); Carmira Farm (this study)	Y	A, B
12.	<i>Hemidactylus benguellensis</i> Bocage, 1893	E	L	Capelongo (Butler et al. 2019)		A
13.	<i>Lygodactylus angolensis</i> Bocage, 1896		RR, V	BNP (this study)	Y	A
14.	<i>Lygodactylus bradfieldi</i> Hewitt, 1932		H, L	Humbe-Cahama, 36 km from; Humbe, 5 km N of; Humbia (Haacke, TM); BNP (Butler et al. 2019)	Y	A, B
15.	<i>Pachydactylus punctatus</i> Peters, 1854 complex		L, V	Kului (Monard 1937b; Bauer et al. 2006); BNP (this study)	Y	A, B

Table 2 (continued). Reptiles recorded from inside and the surroundings of Bicular National Park, Angola, based on historical and recent records. Type of record: CR = new record for the country; H = unpublished record from Ditsong (= Transvaal) Museum's collection; L = literature; O = observation; P = photograph; RR = new record for the region; V = voucher; E = endemic. Period of record: A = after 2008; B = before 1975. Taxonomy has been updated over the years, therefore original species nomenclature may occur under different names.

	Taxa	Endemic?	Type of record	Records in the region of BNP Locality (and Reference)	Inside BNP?	Period of record
16.	<i>Rhoptropus barnardi</i> Hewitt, 1962		H, RR	Chibemba, 11 km S; Chibemba, 5 km S; Humbia, 12 km E (Haacke, TM)		B
Gerrhosauridae						
17.	<i>Gerrhosaurus bulsi-multilineatus</i> complex	E*	H, L, V	Capelongo, Mulondo (Monard 1937b); Cahama, 10 km NW; Cahama, 21 km NW – Chibemba, Cahama, 30 km SE of (Haacke, TM); BNP (Butler et al. 2019; this study)	Y	A, B
18.	<i>Matobosaurus maltzahnii</i> (De Grys, 1938)		H, RR	Dongue, 10 km NW of (Haacke, TM)		B
Lacertidae						
19.	<i>Heliobolus lugubris</i> (Smith, 1838)		H, L	Mulondo (Monard 1937b); Cahama, 3 km NW of (Haacke, TM)		B
20.	<i>Ichnotropis bivittata</i> Bocage, 1866		L	Kului (Monard 1937b)	Y	B
21.	<i>Ichnotropis capensis</i> (Smith, 1838)		L, V	BNP (Butler et al. 2019; this study)	Y	A
22.	<i>Meroles squamulosus</i> (Peters, 1854)		L	Capelongo (Monard 1937b)		B
23.	<i>Nucras broadleyi</i> Branch, Conradie, Vaz Pinto, Tolley 2019	E	L	Capelongo (Monard 1937b)		B
Scincidae						
24.	<i>Acontias occidentalis</i> FitzSimons, 1941		L	Mupa (Monard 1937b; FitzSimons 1941)		B
25.	<i>Mochlus sundevalli</i> (Smith, 1849)		RR, V	Carmira Farm (this study)		A
26.	<i>Panaspis wahlbergi-maculicollis</i> complex		L, V	BNP (Butler et al. 2019; this study)	Y	A
27.	<i>Sepsina angolensis</i> Bocage, 1866		H, L	Chibemba (Laurent 1964); Viriambundo (Haacke, TM)		B
28.	<i>Trachylepis albopunctata</i> (Bocage, 1867)		L	BNP (Butler et al. 2019)	Y	A
29.	<i>Trachylepis binotata</i> (Bocage, 1867)		H, L	Mupa (Monard 1937b); Chibemba, 11 km S; Humbe (Haacke, TM); BNP (Butler et al. 2019)	Y	A, B
30.	<i>Trachylepis chimbana</i> (Boulenger, 1887)		H	Humbe (Schmidt 1933); Humbia, 12 km E of (Haacke, TM)		B
31.	<i>Trachylepis spilogaster</i> (Peters, 1882)		H, L, V	Humbe to Cahama, 36 km NW of (Haacke, TM); BNP (Butler et al. 2019; this study)	Y	A, B
32.	<i>Trachylepis sulcata ansorgii</i> (Boulenger, 1907)		H, RR	Chibemba, 11 km S (Haacke, TM)		B

Herpetofauna of Bicular National Park, Angola

Table 2 (continued). Reptiles recorded from inside and the surroundings of Bicular National Park, Angola, based on historical and recent records. Type of record: CR = new record for the country; H = unpublished record from Ditsong (= Transvaal) Museum's collection; L = literature; O = observation; P = photograph; RR = new record for the region; V = voucher; E = endemic. Period of record: A = after 2008; B = before 1975. Taxonomy has been updated over the years, therefore original species nomenclature may occur under different names.

	Taxa	Endemic?	Type of record	Records in the region of BNP Locality (and Reference)	Inside BNP?	Period of record
33.	<i>Trachylepis sulcata sulcata</i> (Peters, 1867)		L	Capelongo (Butler et al. 2019)		A
34.	<i>Trachylepis varia</i> (Peters, 1867) clade B (Weinell and Bauer 2018)		H, L	Capelongo (Monard 1937b); Cahama, 21 km NW – Chibemba, Humbia, 12 km E of (Haacke, TM)		B
35.	<i>Trachylepis wahlbergi</i> (Peters, 1869)		H, L	Humbi, Capelongo, Kului, Mulondo, Mupa (Monard 1937b); Humbe, 5 km N of (Haacke, TM)		B
Varanidae						
36.	<i>Varanus albigularis angolensis</i> Schmidt, 1933		L, P	Mulondo, Mupa (Monard 1937b); BNP (this study)	Y	A, B
Serpentes						
Colubridae						
37.	<i>Crotaphopeltis hotamboeia</i> (Laurenti, 1768)		L, V	Gambos, Humbe (Bocage 1895); Capelongo (Monard 1937b); Nougafafa Lake (this study)		A, B
38.	<i>Dasypeltis scabra</i> (Linnaeus, 1758)		L	Gambos (Bocage 1895)		B
39.	<i>Dispholidus typus viridis</i> (Smith, 1838)		L, P, V	Humbe (Bocage 1895); Mupa (Monard 1937b); Capelongo (Bogert 1940); Carmira Farm (this study)		A, B
40.	<i>Philothamnus angolensis</i> Bocage, 1882		H, L	Capelongo (Bogert 1940); Humbe (Haacke, TM)		B
41.	<i>Philothamnus semivariatus</i> (Smith, 1840) <i>sensu lato</i>		L, P, V	Humbe (Bocage 1895); Mupa (Monard 1937b); Carmira Farm, Handa Farm (this study)		A, B
42.	<i>Telescopus semiannulatus polystictus</i> Mertens, 1954		L	Humbe, Gambos (Bocage 1895)		B
43.	<i>Thelotornis capensis oatesi</i> (Günther, 1881)		RR, V	BNP (this study)	Y	A
Elapidae						
44.	<i>Dendroaspis polylepis</i> Günther, 1864		L, P	Mulondo (Schmidt 1933); BNP, Handa Farm (this study)	Y	A, B
45.	<i>Elapsoidea semiannulata semiannulata</i> Bocage, 1882		L	Gambos (Bocage 1895; Broadley 1998)		B
46.	<i>Naja anchietae</i> Bocage, 1879		L, V	Humbe (Bocage 1895); Capelongo (Bogert 1940); Mupa (Monard 1937b); BNP, Handa Farm (this study)	Y	A, B

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Taxa	Endemic?	Type of record	Records in the region of BNP Locality (and Reference)	Inside BNP?	Period of record
47. <i>Naja nigricollis</i> Reinhardt, 1843		L, P	Humbe (Bocage 1895); Capelongo (Bogert 1940); Osi (= Osse) (Monard 1937b); Handa Farm (this study)		A, B
Lamprophiidae					
48. <i>Amblyodipsas polylepis</i> (Bocage, 1873)		L	Humbe (Bocage 1895)		B
49. <i>Amblyodipsas ventrimaculata</i> (Roux, 1907)		L, V	BNP (Butler et al. 2019; this study)	Y	A
50. <i>Aparallactus capensis</i> Smith, 1849		L	Gambos (Bocage 1895)		B
51. <i>Boaedon angolensis</i> Bocage, 1895	E	H, L, RR, V	Cahama, 3 km NW of (Haacke, TM); BNP (Butler et al. 2019); Carmira Farm (this study)	Y	A, B
52. <i>Hemirhagerhis viperina</i> (Bocage, 1873)		H, L	Humbe (Bocage 1895); Chibemba, 5 km S (Haacke, TM)		B
53. <i>Prosymna angolensis</i> Boulenger, 1915		L, V	Capelongo (Bogert 1940); BNP (this study)	Y	B
54. <i>Prosymna visseri</i> Fitzsimons, 1959		H, RR	Chibemba, 5 km S (Haacke, TM)		B
55. <i>Psammophis angolensis</i> (Bocage, 1872)		L	Humbe (Bocage 1895; Schmidt 1933)		B
56. <i>Psammophis mossambicus</i> Peters, 1882		L, V	Capelongo (Bogert 1940); Mupa (Monard 1937b); BNP, Handa Farm (this study)	Y	B
57. <i>Psammophis subtaeniatus</i> Peters, 1882		L, P, V	Humbe, Mulondo, Mupa (Monard 1937b); BNP (Butler et al. 2019); Carmira Farm (this study)	Y	A, B
58. <i>Psammophylax tritaeniatus</i> (Günther, 1868)		H, L	Gambos, Humbe (Monard 1937b); Capelongo (Bogert 1940); Humbe–Cahama, 36 km NW of (Hacke, TM)		B
59. <i>Psammophylax ocellatus</i> (Bocage, 1873)	E	L	Gambos, Humbe (Bocage 1895; Branch et al. 2019a)		A, B
60. <i>Pseudaspis cana</i> (Linnaeus, 1758)		L, V	BNP (Butler et al. 2019); Carmira Farm (this study)	Y	A
61. <i>Xenocalamus bicolor bicolor</i> Günther, 1868		CR, V	Carmira Farm (this study)		A
Leptotyphlopidae					
62. <i>Leptotyphlops scutifrons</i> (Peters, 1854)		H, L	Capelongo (Monard 1937b); Quipungo (Haacke, TM)		B
63. <i>Namibiana</i> aff. <i>rostrata</i> (Bocage, 1886)	E	L, V	Humbe (Bocage 1895); BNP (Butler et al. 2019); Handa Farm (this study)	Y	A, B

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Taxa		Endemic?	Type of record	Records in the region of BNP Locality (and Reference)	Inside BNP?	Period of record
Pythonidae						
64.	<i>Python anchietae</i> Bocage, 1887		H, RR	Viriambundo (Haacke, TM)		B
65.	<i>Python natalensis</i> Smith, 1840		L, V	Capelongo (Bogert 1940); BNP (this study)	Y	A, B
Typhlopidae						
66.	<i>Afrotyphlops</i> cf. <i>schlegelii</i> (Bianconi, 1849)		L, P	Humbe (Bocage 1893); BNP (this study)	Y	A, B
Viperidae						
67.	<i>Bitis arietans</i> Merrem, 1820		L, V	Capelongo (Bogert 1940); BNP (this study)	Y	A, B
68.	<i>Causus rhombeatus</i> (Lichtenstein, 1823)		P, RR	Handa Farm (this study)		A
Testudines						
Pelomedusidae						
69.	<i>Pelomedusa subrufa</i> (Bonnaterre, 1789)		L, V	Humbe (Bocage 1895); BNP (Butler et al. 2019; this study)	Y	A, B
70.	<i>Pelusios</i> cf. <i>nanus</i> Laurent, 1956		L	Osi (= Osse) (Monard 1937b); Broadley (1981)		B
Testudinidae						
71.	<i>Kinixys</i> cf. <i>belliana</i> Gray, 1831		L, V	Osi (= Osse) (Monard 1937b); BNP (Butler et al. 2019; this study)	Y	A, B
72.	<i>Stigmochelys pardalis</i> (Bell, 1828)		L	Mupa (Monard 1937b)		B
Crocodilia						
Crocodylidae						
73.	<i>Crocodylus niloticus</i> Laurenti, 1768		L, O	Capelongo (Monard 1937b); BNP (this study)		A, B

Comment: *Monopeltis anchietae* is recorded from southwestern Angola (Marques et al. 2018), and Branch et al. (2019b) discuss its taxonomical history. The coloration pattern of the specimen (Fig. 13) conforms to the most common pattern associated with *M. anchietae* (Broadley et al. 1976), but the photograph resolution does not allow for the detailed scale counts necessary to provide a positive identification. The species was recorded in Humbe (Bocage 1873), approximately 110 km south of BNP, and the Carmira Farm specimen is provisionally assigned to *M. anchietae*, pending the collection of additional material for confirmation.

Monopeltis infuscata Broadley, 1997
Infusate Spade-snouted Worm Lizard (Fig. 14)
Material: One unlabelled and bleached specimen in Carmira Farm’s private collection.

Comment: Scallation features of the specimen (dorsal head shield with blind lateral sutures (Fig. 14), four postgenials in the first row, more than seven postgenials in the second row, two precloacal pores, 204 body annuli, and 10 caudal annuli) match the description of *M. infuscata* (Broadley 1997). In Angola, the species was recorded in Humbe in Cunene Province, Tombole River in Cuando-Cubango Province, and a locality named “Sturuba” (Broadley 1997) which could not be determined. This is the third confirmed locality for the species in the country and the first for Huíla Province, and additional voucher specimens and genetic material should be collected. Found among sandy soils in *Baieka/Burkea* woodlands, *Monopeltis anchietae* and *M. infuscata* are known to be sympatric in Humbe (Broadley 1997), approximately 110 km south of BNP, supporting their co-occurrence in Carmira Farm.



Fig. 12. *Acanthocercus* sp., BNP (male).

Zygaspis quadrifrons (Peters, 1862)

Kalahari Round-snouted Worm Lizard (Fig. 15)

Material: One unlabelled and bleached specimen in Carmira Farm's private collection.

Comment: This specimen conforms morphologically to *Z. quadrifrons* (Broadley and Broadley 1997). For many years, the species was known from Angola only from Monard's (1931) description of *Amphisbaena ambuellensis* from 'Chimporo' (= Tchimpolo), which was subsequently synonymized with *A. quadrifrons* by Loveridge (1941) and later transferred to *Zygaspis* by Alexander and Gans (1966). No additional Angolan material was collected until recent surveys in southeastern Angola (Conradie et al. 2016) and BNP (Butler et

al. 2019). Broadley and Broadley (1997) recognized five groups within *Z. quadrifrons*. Additional voucher specimens and genetic material are necessary to confirm species identification, and to evaluate the validity of Monard's taxon "*ambuellensis*" for Angolan material (Branch et al. 2019c). Found in the same habitat as *M. infuscata*.

Chamaeleonidae

Chamaeleo dilepis quilensis (Bocage, 1886)

Quilo Flap-necked Chameleon (Fig. 16)

Material: NB760 (33); NB 1084 (C1).

Comment: This subspecies is common and widespread throughout Angola (Marques et al. 2018). It was recently recorded in BNP (Butler et al. 2019) and observed throughout the park, and it has been historically recorded near BNP (Monard 1937b; Haacke, TM).

Gekkonidae

Chondrodactylus aff. *laevigatus* (Fischer, 1888)

Button-scaled Gecko

Comment: A large individual of the genus *Chondrodactylus* was observed on buildings at Carmira Farm (N. Baptista, pers. obs.), but it was not photographed or collected, and is provisionally assigned to *C. laevigatus* based on recent records of this species from BNP (Butler et al. 2019). Records from near the park in Mulondo (Schmidt 1933, as *Pachydactylus stellatus*), and Humbe (Monard 1937b, as *P. bibroni*) are also assigned to this taxon, which is known to occur in southwestern Angola (Marques et al. 2018).

Lygodactylus angolensis Bocage, 1896

Angola Dwarf Day Gecko (Fig. 17)

Material: NB517 (31).

Comment: Specimens of this taxon were first described as *L. capensis* by Bocage (1895), who subsequently assigned them to a new species, *L. angolensis*, described



Fig. 13. *Monopeltis* cf. *anchietae*, Carmira Farm (Photo by L. Gata).



Fig. 14. *Monopeltis infuscata*, Carmira Farm.



Fig. 15. *Zygaspis quadrifrons*, Carmira Farm.

from Hanha, Benguela Province (Bocage 1897). Schmidt (1933) described a new species of dwarf day gecko, *L. laurae*, which was later synonymized with *L. angolensis* (Marques et al. 2018; Branch et al. 2019c). Widely distributed through Africa, localities in Angola were compiled by Marques et al. (2018) and include Chimboa da Hanha (= Capira); Cacondo (= Caconda), 2 km W of; Cubal; Cutenda, 3 km S of; Negola, 6 km S of; and Quimbango (Haacke, TM). The single BNP specimen conforms to the original description of *L. angolensis* in scalation and coloration, and is similar to specimens collected in the urban environments of Lubango (Baptista, unpub. data). Specimens collected at the same localities (BNP and Lubango) on other occasions have been assigned to *L. bradfieldi* (Butler et al. 2019), and these observations deserve further morphological and genetic comparisons.

Pachydactylus punctatus Peters, 1854 complex
Speckled Thick-toed Gecko (Fig. 18)

Material: NB513 (14); NB514 (T1); NB515 (T1); NB530 (T3); NB773 (34); NB774 (34); NB775 (34).

Comment: This species was recorded in southwestern Angola (Monard 1937b; Laurent 1964; Marques et al. 2018), including Kului, near BNP (in Monard 1937b as *P. serval*, assigned to *P. punctatus* by Bauer et al. 2006). BNP specimens are morphologically different



Fig. 16. *Chamaeleo dilepis quilensis*, BNP.

(larger, sturdier, paler, and with a different blotching pattern) from other specimens found in Tundavala and Lubango, Huíla Province (Baptista et al. 2018; Butler et al. 2019), that are also assigned to the *P. punctatus* complex. These lizards also inhabit different habitats and microhabitats (found on the ground in rocky areas in Tundavala; under the bark of fallen logs in recently burnt areas during the day and in leaf litter near a dirt road during the night in BNP) and very likely belong to different species. Tundavala is along the high altitude edge of the Angolan plateau (above 2,000 m asl) and, given the very specious nature of this genus (Heinicke et al. 2017) and the existence of cryptic diversity reported in Angola (Branch et al. 2017), the taxonomic status of this complex requires further investigation.

Gerrhosauridae

Gerrhosaurus bulsi-multilineatus complex
Plated Lizard (Fig. 19)

Material: NB531 (31); NB776 (2); NB777 (3).

Comment: Five species of *Gerrhosaurus* are known to exist in Angola: *G. auritus*, *G. bulsi*, *G. multilineatus*, *G. nigrolineatus*, and *G. skoogi* (Branch et al. 2019c). *Gerrhosaurus nigrolineatus* is recorded in areas adjacent to BNP, in Capelongo and Mulondo (Monard 1937b), and from the surroundings of Cahama (Haacke, TM).



Fig. 17. *Lygodactylus angolensis*, BNP.



Fig. 18. *Pachydactylus punctatus* complex, BNP.



Fig. 19. *Gerrhosaurus bulsi-multilineatus* complex (juvenile), BNP.

Bates et al. (2013) discussed the problematic status of Angolan populations referred to as *G. nigrolineatus*, and species boundaries within the *G. bulsi-multilineatus* complex remain unresolved. Assignment of recently collected specimens is pending until genetic assessment of the Angolan material is published (M. Bates, pers. comm.). Specimens collected recently in BNP by Butler et al. (2019) were assigned to *G. cf. multilineatus*. One juvenile was collected in the Main Camp (Fig. 19), and adults were collected from burrows in degraded shrubland near the park boundary, and they are similar to the specimen illustrated in Butler et al. (2019).

Lacertidae

Ichnotropis capensis (Smith, 1838)

Cape Rough-scaled Lizard (Fig. 20A–B)

Material: NB771 (34); NB772 (34); NB779 (12); photographic record (M. Finckh, site 43).

Comment: *Ichnotropis capensis* and *I. bivittata* are known to occur sympatrically in Angola (Laurent 1964; Marques et al. 2018), and five taxa within this genus are listed for Angola (Marques et al. 2018; Branch et al. 2019c). The systematics of the genus *Ichnotropis* is poorly established (Laurent 1964), and while there is no recent systematic revision of the group (Edwards et al. 2013), a thorough historical revision was recently published (Berg 2017). According to this, two subspecies of *I. capensis* occur in Angola, *I. c. capensis* (Smith, 1838) and *I. c. overlaeti* Witte and Laurent, 1942, with the latter being restricted to northern Angola (Marques et al. 2018). Rough-scaled lizards have recently been collected in BNP and were referred to *Ichnotropis bivittata pallida* Laurent, 1964 (Butler et al. 2019), but we have regarded them as *I. capensis*, given that *I. b. pallida* is morphologically distinct, and occurs in higher altitudes, such as Humpata (Laurent 1964). Specimens from BNP have a bright orange/red lateral line that is more evident in males than in females, and living males have a bright yellow chin and chest (Fig. 20A–B) that becomes bleached when preserved. Detailed and comprehensive studies of species within this genus in Angola are needed.



Fig. 20. *Ichnotropis capensis*, BNP. (A) Female; (B) male.

Scincidae

Mochlus sundevalli (Smith, 1849)

Sundevall's Writhing Skink (Fig. 21)

Material: One unlabelled bleached specimen in Carmira Farm's private collection.

Comment: Widespread throughout eastern and southern Africa, in Angola this species is recorded from the coastal plains south of Cuanza River, lower Cuando River basin (records compiled in Marques et al. [2018]), and northeastern Angola in Dundo (Laurent 1964).

Panaspis wahlbergi-maculicollis complex

Snake-eyed Skink (Fig. 22A–B)

Material: NB516 (T3); NB548 (T1); NB549 (T2).

Comment: Small leaf-litter inhabiting skinks have numerous cryptic lineages in southern and eastern Africa (Medina et al. 2016). Historically, Bocage (1895) reported on material from Caconda and Cahata collected by Anchieta, and recently *P. maculicollis* was recorded from southeastern Angola (Conradie et al. 2016). A population of "*P. wahlbergi*" in northern Namibia was shown to form part of the *P. maculicollis* complex (Medina et al. 2016) and was subsequently described as a new species, *Panaspis namibiana* (Ceríaco et al. 2018a). Snake-eyed skinks recently collected in BNP were assigned to *P. aff. namibiana* (Butler et al. 2019). BNP specimens from this study have fused anterior parietals, conforming to the *P. wahlbergi* complex, but prefrontals are well separated (see Fig. 22B), distinguishing them from *P. namibiana* (Ceríaco et al. 2018a). The taxonomic status of the BNP



Fig. 21. *Mochlus sundevalli*, Carmira Farm.

population and its affinities to the *P. maculicollis* or *P. wahlbergi* radiations requires further study, as do other Angolan populations from Humpata, Quilengues, and the Cuanza Sul escarpment (Vaz Pinto and Baptista, unpub. data).

Trachylepis spilogaster (Peters, 1882)

Kalahari Tree Skink

Material: NB519 (31); NB527 (10); NB528 (9); NB529 (13); NB532 (6); NB533 (9); NB778 (31).

Comment: Recently recorded in BNP (Butler et al. 2019), it was the most frequently observed reptile species during the BNP surveys, and together with *Pachydactylus punctatus*, was one of only two species to be found in the park's woodlands after recent fires.

Varanidae

Varanus albigularis angolensis Schmidt, 1933

Angolan Savanna Monitor (Fig. 23)

Material: One photographic record (M. Finckh, site 40).

Comment: Several records of *Varanus albigularis* from Angola have been assigned to two different subspecies (Marques et al. 2018). Schmidt (1933) described *V. a. angolensis* from 'Gaúca, Bihe' (= Zaúca River, Malanje) (Crawford-Cabral and Mesquitela 1989), and the BNP record is assigned to this taxon. This species still needs a wide-ranging phylogenetic assessment. The individual was observed in the ecotone between grassland and woodlands.

Serpentes

Colubridae

Crotaphopeltis hotamboeia (Laurenti, 1768)

White-lipped Herald Snake

Material: NB522 (41); four unlabelled specimens in Carmira Farm's private collection; one photographic record from Handa Farm (J. Traguedo, HF).

Comment: A widespread and common species in Angola (Marques et al. 2018), found active at night, on the edge of Lagoa Nougafafa (= Nugarrafa, = Nongafafa).



Fig. 22. *Panaspis wahlbergi-maculicollis* complex, BNP. Head (A) lateral view and (B) dorsal view.

Dispholidus typus viridis (Smith, 1838)

Green Boomsnake (Fig. 24)

Material: Two unlabelled bleached juvenile specimens in Carmira Farm's private collection; one photographic record (L. Gata, CF).

Comment: Branch (2018) provides an overview of the two subspecies of boomslang existing in Angola, *D. t. punctatus* and *D. t. viridis*. These were shown to deserve full species status (Eimermacher 2012), but this taxonomic adjustment requires further consensus. Neither scalation nor juvenile coloration allow assignment of the Carmira Farm specimens to either taxon, but the green coloration of a photographed adult (Fig. 24) is consistent with *D. t. viridis* (Eimermacher 2012), as are other material from Humbe. Resolution of species boundaries in *Dispholidus* and the assignment of Angolan populations requires further genetic studies.

Philothamnus semivariegatus (Smith, 1840) *sensu lato*

Spotted Bush Snake (Fig. 25)

Material: Two unlabelled bleached specimens in Carmira Farm's private collection; one photographic record from Handa Farm (J. Traguedo, HF).

Comment: The complex taxonomic history of this species is discussed by Branch (2018). We cautiously assign specimens from Carmira Farm to *P. semivariegatus* based on scalation (three supra-labials entering orbit, temporal arrangement 2+2, and keeled ventrals), however, coloration of specimens from both farms did not have any markings (atypical of *P. semivariegatus*).



Fig. 23. *Varanus albigularis angolensis*, BNP (Photo by M. Finkch).

Historical records from Angola were compiled (Marques et al. 2018), and additional records include coastal lowlands in Benguela Province (Vaz Pinto, unpub. data). This species is paraphyletic having at least four different lineages (Engelbrecht et al. 2019), and the Carmira Farm specimens might group with clade 4. Records of *P. angolensis* from Capelongo (Bogert 1940) and Humbe (Haacke, TM) deserve careful comparison with this material.

Thelotornis capensis oatesi (Günther, 1881)

Oates' Vine Snake (Fig. 26)

Material: NB1065 (5).

Comment: Widely distributed in Angola, with records from Hanha (Bogert 1940), Chitado (Hellmich 1957), Alto Chicapa (Laurent 1964), and Longa (Conradie et al. 2016). The specimen collected in BNP conforms in coloration and scalation to this subspecies (see Fig. 26).

Elapidae

Dendroaspis polylepis Gunther, 1864

Black Mamba

Material: Shed skin found in BNP. Photographic records (L. Gata, CF; J. Traguedo, HF).

Comment: This species is recorded from several



Fig. 25. *Philothamnus semivariiegatus sensu latu*, Carmira Farm.



Fig. 24. *Dispholidus typus viridis*, Carmira Farm (Photo by L. Gata).

localities in Angola (Marques et al. 2018). Locally known as “kuiva” (J. Traguedo, pers. comm.).

Naja anchietae Bocage, 1879

Anchieta's Cobra (Fig. 27A–B)

Material: NB793 (21); NB250 (banded morph) [HF].

Comment: Branch (2018) discusses the description, history, and taxonomy of *N. anchietae* in Angola. This is a common species occurring in southern Angola, with known records compiled by Broadley (1995). Recent records are from Malanje Province (Ceriaco et al. 2014, Vaz Pinto, unpub. data), on the edge of the plateau in Tundavala (Baptista et al. 2018), Cassinga (Vaz Pinto, unpub. data), and Bimbe (Baptista and Vaz Pinto, unpub. data).

Naja nigricollis Reinhardt, 1843

Black-necked Spitting Cobra (Fig. 28)

Material: Photographic record (J. Traguedo, HF).

Comment: The taxonomy of African cobras (*Naja*) has undergone significant changes in recent years (Branch 2018). The name *Naja nigricollis* has a complex history of synonymies and varieties, and only when two varieties, *N. n. nigricincta* and *N. n. woodi*, were elevated to full species, was the name *N. nigricollis* stabilized to represent a species (Wuster et al. 2007). It is widespread



Fig. 26. *Thelotornis capensis oatesi*, BNP (Photo by T. António).



Fig. 27. *Naja anchietae*. (A) BNP (Photo by F. Maiato); (B) Handa Farm (Photo by J. Traguedo).

in Angola, but avoids dense forest. Locally known as “turula n’jila” (J. Traguedo, pers. comm.).

Lamprophiidae

Amblyodipsas ventrimaculata (Roux, 1907)

Kalahari Purple-glossed Snake

Material: NB595 (20); one individual seen in grasslands near lake from Main Camp (site T3), not collected.

Comment: The specimen’s scalation (15 midbody scale rows, 21 subcaudals, 203 ventrals, five upperlabials, 2nd and 3rd entering the eye) and coloration (similar to specimen illustrated in Butler et al. [2019]) conform to *A. ventrimaculata* (Branch 1998; Marais 2004). Together with Butler et al. (2019), BNP is the second record of this species for Angola, the first being from the Cuito River source (Portillo et al. 2018; Branch et al. 2019c), and represents a northwestern extension of the known range (Botswana, Zimbabwe, Namibia, and Zambia).

Boaedon angolensis (Bocage, 1895)

Angolan House Snake (Fig. 29A–B)

Material: One unlabelled bleached specimen in the private collection of Carmira Farm.

Comment: This species was recently recorded from BNP (Butler et al. 2019). The scalation of the Carmira Farm specimen (23 midbody scale rows, 116 ventrals, more than 55 subcaudals, see Fig. 29A–B for head scalation), accords with the revision of Angolan house snakes currently in progress (Hallermann et al., unpub. data).



Fig. 28. *Naja nigricollis*, Handa Farm (Photo by J. Traguedo).

Prosymna angolensis Boulenger, 1915

Angolan Shovel-snout Snake (Fig. 30)

Material: NB521 (T1).

Comment: Originally described from Huila, Angola (discussion on type locality in Branch [2018]), this species was recorded historically from southwestern and central Angola (Marques et al. 2018), and more recently, from the southeast of Angola (Conradie et al. 2017) and Tundavala (Baptista, unpub. data). Its upper and lower labials, ventral scales (145), and low subcaudal counts (16) conform to the species characterization (Bocage 1895, as *P. frontalis*). It has gray coloration with a dark blotch behind the head, a series of paired dark spots along the back, and immaculate white/creamish belly and flanks. It was found in sandy soils in miombo woodlands.

Psammophis mossambicus Peters, 1882

Olive Grass Snake (Fig. 31)

Material: NB 518 (head only) (T1); photographic record (J. Traguedo, HF).

Comment: This species belongs to the *P. sibilans* complex (Kelly et al. 2008, Trape et al. 2019). The collected snake had a uniformly pale-yellow belly, and dorsal coloration was plain gray with a thin darker vertebral dash. Head scalation and ventral (159) and subcaudal (80) scale counts recorded from the specimen conform to *P. mossambicus* (Broadley 2002, Trape et al. 2019). It is locally known as “muiha on njolo” (J. Traguedo, pers. comm.).

Psammophis subtaeniatus Peters, 1882

Stripe-bellied Sand Snake (Fig. 32)

Material: Four unlabelled specimens from Carmira Farm private collection; one photographic record (L. Gata, CF).

Comment: This species is restricted to the semi-arid scrubland and mopane woodland, above and below the escarpment in southwestern Angola (Branch 2018). Additional recent records include specimens from Equimina and Serra da Neve (Vaz Pinto and Baptista, unpub. data).



Fig. 29. *Boaedon angolensis*, Carmira Farm. Head (A) lateral view and (B) dorsal view.

Pseudaspis cana (Linnaeus, 1758)

Mole Snake

Material: One unlabelled specimen from Carmira Farm private collection; one photographic record (L. Gata, CF).

Comment: This snake is known from several localities in Angola (Branch 2018; Marques et al. 2018) and was recently found in BNP (Butler et al. 2019).

Xenocalamus bicolor bicolor Günther, 1868

Slender Quill-snouted Snake (Fig. 33A–B)

Material: Two unlabelled specimens in the private collection of Carmira Farm.

Comment: *Xenocalamus bicolor* is only recorded in Angola from a northern subspecies described in the northeast of the country, *X. b. machadoi* (see Broadley 1971; Branch 2018; Marques et al. 2018). It occurs in the Zambezi Region and adjacent western Zambia, and it is usually associated with Kalahari sands (Branch et



Fig. 30. *Prosymna angolensis*, BNP.

al. 2019). Its presence was considered to be likely for southeastern Angola (Branch et al. 2019), and this record is the first for the country. The presence of supraoculars separating the frontals from contacting the orbit (Fig. 33B), conforms to *X. b. bicolor*, as does the number of ventrals (204, 233) [Broadley 1971]. *Xenocalamus b. bicolor* is known to have great intraspecific variation in coloration, and this feature is rarely diagnostic (Broadley 1971). The patterns of both Carmira Farm specimens resemble the “*maculatus*” phase described by Broadley (1971), but more heavily marked (Fig. 33A).

Leptotyphlopidae

Namibiana aff. *rostrata* (Bocage, 1886)

Angolan Beaked Thread Snake (Fig. 34)

Material: NB599 (HF).

Comment: Thread snakes are a difficult group to study due to their very small size and conservative morphology (Broadley and Broadley 1999; Branch 2018). *Namibiana rostrata* is endemic to Angola, and originally described from Humbe, ca. 110 km south of BNP. It has recently been recorded from BNP (Butler et al. 2019), and we provisionally assign the specimen from Handa Farm to the same species. Haacke (TM) collected two thread snakes, one from Paiva Couceiro (= Quipungo, TM45190), around 30 km southeast of Handa Farm, and another from Hoque (TM 46699), ca. 77 km northwest of BNP,



Fig. 31. *Psammophis mossambicus*, Handa Farm (Photo by J. Tragudo).

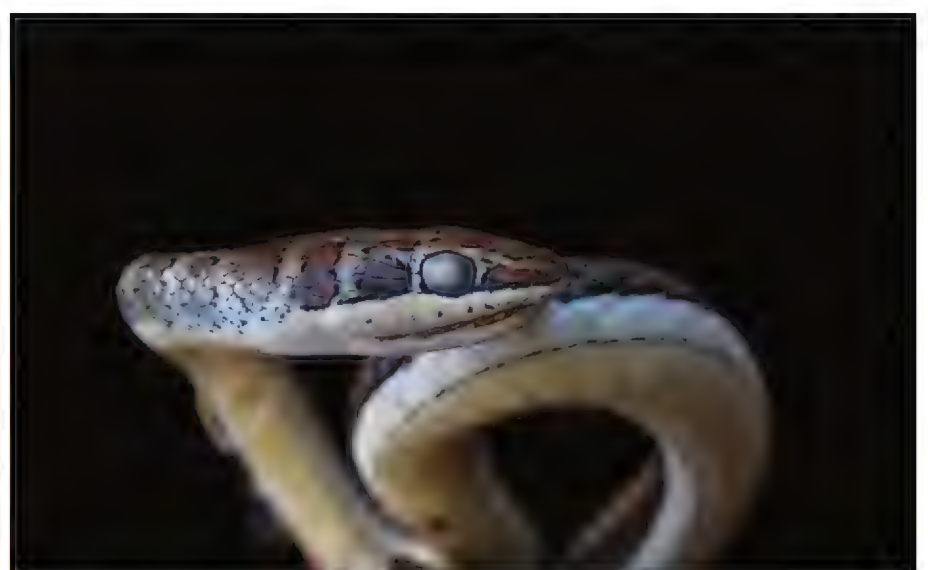


Fig. 32. *Psammophis subtaeniatus*, Carmira Farm.

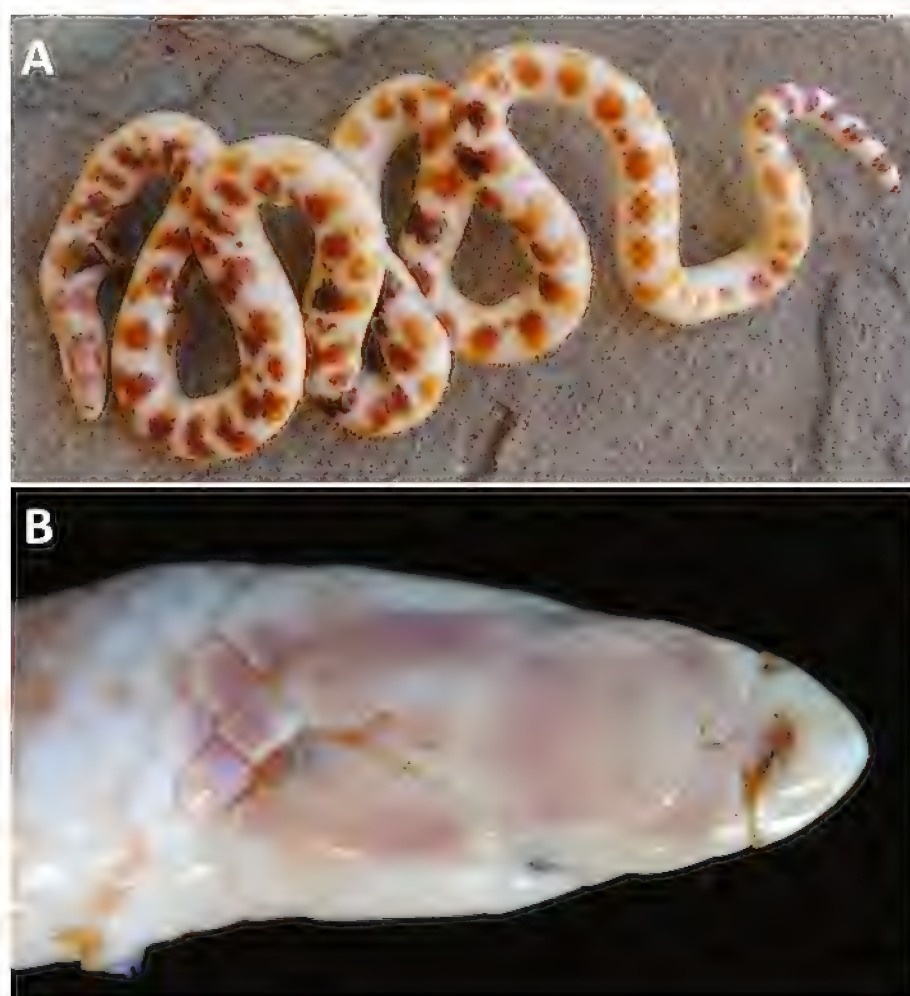


Fig. 33. *Xenocalamus bicolor bicolor*, Carmira Farm. (A) Full specimen; (B) dorsal view of head.



Fig. 34. *Namibiana* aff. *rostrata*, Handa Farm.

both identified as *Leptotyphlops scutifrons scutifrons*. All these records should be carefully compared to confirm species identification.

Pythonidae

Python anchietae Bocage, 1877

Namib Dwarf Python

Comment: *Python anchietae* is endemic to the Namib Desert and adjacent areas in Angola and Namibia. It was collected in Viriambundo (Haacke, TM), ca. 30 km west of BNP. Although this is a historical record, it is discussed in the species accounts here due to its relevance. This is the fourth known record of this near-endemic species in the country, after the type locality Catumbela (Bocage 1887), Hanha (Bogert 1940), and between Lobito and Hanha (Laurent 1964), and the first record above the Angolan escarpment. In Namibia, it occurs inland up to near Windhoek, indicating that the species may extend further inland in Angola and occur in BNP.



Fig. 35. *Afrotyphlops* cf. *schlegelii*, BNP (Photo by M. Finkch).

Python natalensis Smith, 1840

Southern African Python

Material: NB794 (1); one individual observed in BNP's hide for game viewing (27); one photographic record from Carmira Farm (L. Gata, CF); several photographic records from Handa Farm (J. Traguado, HF).

Comment: Two species of African python exist in Angola, the northern *P. sebae*, and the southern *P. natalensis* (Branch 2018). Material from BNP belongs to *P. natalensis*.

Typhlopidae

Afrotyphlops cf. *schlegelii* (Bianconi, 1849)

Schlegel's Blind Snake (Fig. 35)

Material: Photographic record (M. Finckh, 39).

Comment: One specimen was photographed after heavy rainfall at night (nearly 100 mm of precipitation). Dorsal coloration was dark yellowish with scattered dark blotches (Fig. 35) and snout-vent length measured around 70 cm (M. Finckh, pers. comm.). *Afrotyphlops schlegelii* is a sister species of *A. mucruso*, and both have been recorded from Angola. Marques et al. (2018) limit *A. mucruso* to northeastern Angola, and *A. schlegelii* to the southwest, and Haacke (TM) also has records for Huíla Province (as *Rhinotyphlops schlegelii petersii*). A taxonomic discussion is presented in Broadley and Wallach (2009), and further discussions of both species in Angola are presented by Conradie et al. (2016) and Branch (2018). Confident identification requires additional material, and the genus deserves further taxonomic revisions.

Viperidae

Bitis arietans Merrem, 1820

Puff Adder

Material: NB713 (19); one photographic record and one specimen from Carmira Farm private collection.

Comment: Common and widespread in Angola, recorded from several localities (Bocage 1895; Ferreira 1897; Schmidt 1933; Bogert 1940; Hellmich 1957; Laurent 1950, 1954, 1964; Thys van der Audenaerde 1967; Manaças 1981; Conradie et al. 2016), with few



Fig. 36. *Causus rhombeatus*, Handa Farm (Photo by J. Traguedo).

records from the southwest (Branch 2018; Marques et al. 2018). In Angola, it is widely known as “surucucu.”

Causus rhombeatus (Lichtenstein, 1923)

Rhombic Night Adder (Fig. 36)

Material: Photographic record from Handa Farm (J. Traguedo, HF).

Comment: The taxonomy of night adders in Angola has long been confusing (Branch 2018). *Causus bilineatus* was described from a variety of *C. rhombeatus* by Boulenger (1905), but synonymies and species validity remained problematic until a recent revision by Rasmussen (2005), who reassessed *C. bilineatus* and related species, mapped the geographic distribution of Angolan night adders, and provided a key to the genus. Scale counts could not be made on the specimen from Handa Farm, although the photographed snake had a very conspicuous V-shaped marking on the head, 25 well-separated dark conspicuous rhombic vertebral markings occasionally with a white contour against a paler background, and lacked dorsolateral stripes (Fig. 36). It was therefore assigned to *C. rhombeatus*, which is widespread in Angola (Rasmussen 2005; Marques et al. 2018).

Testudines

Pelomedusidae

Pelomedusa subrufa (Bonnaterre, 1789)

Marsh Terrapin

Material: NB780 (35); photographic record (M. Finckh, site 43).

Comment: This species is widespread in southern, eastern, and western Africa (Branch 2012; Turtle Taxonomy Working Group 2017). It has been recorded from several localities in Angola (Marques et al. 2018), and recently from BNP (Butler et al. 2019). Petzold et al. (2014) recorded unexpected species diversity in

a molecular phylogeny of *Pelomedusa* and referred northern Namibian material to *P. subrufa*.

Testudinidae

Kinixys cf. *belliana* Gray, 1831

Bell's Hingeback Tortoise

Material: NB711 (18); NB712 (17); NB509 (16) (tissue only); photographic record (M. Finckh, site 43).

Comment: In a molecular phylogeny of *Kinixys*, only *K. belliana* was shown to occur in Angola (Kindler et al. 2012). However, only northern Angolan material was included and it is very likely that *K. spekii* may enter southern Angola (see Marques et al. 2018), thus the identification as *K. belliana* is tentative. This group is widely distributed in Angola (Marques et al. 2018) and has recently been collected in BNP (Butler et al. 2019). This species has been observed at several sites throughout the park and is locally harvested for consumption and for the pet trade.

Crocodylia

Crocodylidae

Crocodylus niloticus Laurenti, 1768

Nile Crocodile

Material: Interviews.

Comment: Staff from BNP referred to the existence of this species in the Cunene River, the eastern boundary of the park. Historical records mention its occurrence in the area (Monard 1937b; Simões 1971).

Discussion

This account presents one new record for the country (*Xenocalamus bicolor bicolor*), as well as records of reptile species that are potentially new to science (*Pachydactylus*, *Namibiana*). Butler et al. (2019) reported eight amphibian taxa and 21 reptile taxa (two testudines, 14 lizards, and five snakes) from BNP and adjacent Capelongo, while this study doubles the number of amphibian species (16), and almost quadruples the number of snake species (18) encountered. These increases in known species diversity are more significant when compared to the total species counts (94, including historical records) for the region. Despite the considerable update in knowledge that this study presents, further surveys are likely to describe additional diversity. Closely related taxa recorded in this study and by Butler et al. (2019), namely *Lygodactylus angolensis* and *L. bradfieldi*, require further research and comprehensive comparisons to confirm whether they refer to sympatric congeneric species or different designations of the same taxa.

Ambiguous taxonomy reflects the unresolved status of Angolan herpetology, as the bulk of species accounts

presented here refer to problematic taxonomy and a lack of supporting studies. This is true even for the BNP region, which is situated within the southwest, the more thoroughly studied part of the country. This knowledge gap reinforces the importance of promoting surveys which include voucher specimens and DNA sample collections published with survey results, as highlighted previously (Russo et al. 2019). The inclusion of DNA barcoding for identification purposes is increasingly common (Bittencourt-Silva 2019) and provides a more comprehensive understanding of the results. Although not included in the scope of this publication, biopsies and specimens collected are being used for ongoing studies (Baptista et al., unpub. data; Lobon-Rovira et al., unpub. data).

The herpetofauna of the BNP region is similar to that of other regions further east where Kalahari sands are also the dominant substrate. It shares 12 amphibian and 30 reptilian species with southeastern Angola (Conradie et al. 2016); seven amphibian and 20 reptilian species with western Zambia, Ngoye Falls, and surrounding areas (Pietersen et al. 2017); and nine amphibian and five reptilian species with recently surveyed areas of west Zambia (Bittencourt-Silva 2019).

In a study of herpetofauna in the southern Kalahari domain, Haacke (1984) noted the relevance of a latitudinal rainfall gradient as a driver of diversity in the Kalahari. This gradient affects vegetation canopy structure on a wide scale in Botswana (Scholes et al. 2004), and it is also reflected in the avifauna species assemblages of BNP, which comprise biome-restricted species from both the northern Angolan Miombo Woodland and the southern Zambezian Baikiaea Woodland ecoregions (Dean 2000; BirdLife International 2018). The effect of this gradient on the herpetofauna of BNP is not yet known, and would be an interesting research topic for understanding the relevance of BNP for conserving herpetofauna. Records of species that were previously only reported from the coastal plain of Angola, such as *Python anchietae*, have shown that they occur above the escarpment. This shows how studying the park's herpetofauna may also contribute to understanding the west-to-east altitudinal and rainfall gradients, and the distribution limits of plateau and lowland species.

Monopeltis perplexus, an endemic Angolan amphisbaenian, was originally described from a vague type locality: "Hanha or Capelongo" (see discussion in Branch et al. 2018). If Capelongo (9 km northeast of BNP) is confirmed as the type locality, *M. perplexus* is likely to occur in BNP and would be sympatric with *M. anchietae* and *M. infuscata*. Sympatric distributions of amphisbaenians highlight the importance of Kalahari sands for fossorial species. Although fossorial species are well represented in the BNP region, the absence of fossorial groups such as *Acontias*, *Typhlacontias*, and *Sepsina*, which have been recorded from adjacent areas on Kalahari sands (Monard 1937b; Branch and

McCartney 1992; Haacke 1997; Conradie et al. 2016; Pietersen et al. 2017; Marques et al. 2018; Bittencourt-Silva 2019) is likely a result of undersampling.

Rupicolous species such as *Cordylus machadoi*, *Matobosaurus maltzahnii*, *Hemirhagerrhis viperina*, and *Agama p. schaki* were not found in the park despite historical records, likely as a reflection of the rarity of rocky outcrops. Substrate specificity and isolation have important influences on the geographic distribution of reptiles, especially lizards, and are considered better determinants of presence than vegetation type (Bauer and Lamb 2005; Roll et al. 2017). Endemic sand-adapted reptiles were reported in the Kalahari (Haacke 1984). The BNP region has a small contact zone with the larger Kalahari sands block to the east (Missão de Pedologia de Angola 1959; Huntley 2019). Moreover, the park is delimited in the east by the large Cunene River, which may be a barrier to species dispersal, much like the Zambezi River (Pietersen et al. 2017). The combination of factors driving speciation in the BNP region, especially for fossorial species, is thus still unclear, and may be clarified by assessing the genetic divergence between BNP specimens and those from the easternmost regions of the Kalahari sands (e.g., southeastern Angola).

Commercial and small-scale farms occupy large portions of northwest BNP (Mendelsohn and Mendelsohn 2018). Additional disturbances include poaching and conversion of woodlands into thickets and shrubland following intense and frequent fires (Mendelsohn and Mendelsohn 2018; Mendelsohn 2019). Several mammalian species are either extinct or on the verge of extinction in the park (Overton et al. 2017; Mendelsohn and Mendelsohn 2018), but the consequences of these disturbances for herpetofauna are largely unknown. Some species are reportedly collected opportunistically for the pet and bushmeat trades (e.g., *Kinixys*), a phenomenon that has also occurred in Cangandala National Park (Ceriaco et al. 2018c).

The establishment of protected areas generally favors the conservation of mammals, birds, and amphibians, while reptiles are usually neglected (Roll et al. 2017). Likewise, the distributions of large mammals in Angola has defined the designation of conservation areas (Huntley et al. 2019a). BNP is an Important Bird and Biodiversity Area, relevant for the conservation of avifauna (Dean 2000; BirdLife International 2018) that also supports considerable reptile diversity. Some of the species likely to occur in the park are endemic to Angola (*Monopeltis perplexus*, *Nucras broadleyi*, *Psammophylax ocellatus*, *Namibiana rostrata*), to Angola and Namibia (*Python anchietae*), or are poorly studied (*Mertensophryne mocquardi*). A better understanding of the park's herpetofauna should reinforce its important role in the conservation of Angolan biodiversity.

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Literature Cited

- Alexander AA, Gans C. 1966. The pattern of dermal-vertebral correlation in snakes and amphisbaenians. *Zoologische Mededelingen* 41(11): 171–190.
- Amiet JL. 2005. Les *Hyperolius* camerounais du groupe d'*H. nasutus* (Amphibia, Anura, Hyperoliidae). *Revue Suisse de Zoologie* 112(1): 271–310.
- Baptista N, António T, Branch WR. 2018. Amphibians and reptiles of the Tundavala region of the Angolan escarpment. *Biodiversity & Ecology* 6: 397–403.
- Baptista N, Conradie W, Vaz Pinto P, Branch WR. 2019. The amphibians of Angola: early studies and the current state of knowledge. Pp. 243–281 In: *Biodiversity of Angola. Science & Conservation: A Modern Synthesis*. Editors, Huntley BJ, Russo V, Lages F, Ferrand N. Springer, Cham, Switzerland. 549 p.
- Barbosa LAG. 1970. *Carta Fitogeográfica de Angola*. Instituto de Investigação Científica de Angola, Luanda, Angola. 323 p.
- Bates MF, Branch WR, Bauer AM, Burger M, Marais J, Alexander GJ, De Villiers MS. 2014. *Atlas and Red List of the Reptiles of South Africa, Lesotho, and Swaziland*. South African National Biodiversity Institute, Pretoria, South Africa. 487 p.
- Bates MF, Tolley KA, Edwards S, Davids Z, Da Silva JM, Branch WR. 2013. A molecular phylogeny of the African plated lizards, genus *Gerrhosaurus* Wiegmann, 1828 (Squamata: Gerrhosauridae), with the description of two new genera. *Zootaxa* 3750(5): 465–493.
- Bauer AM, Lamb T. 2005. Phylogenetic relationships of southern African geckos in the *Pachydactylus* group (Squamata: Gekkonidae). *African Journal of Herpetology* 54(2): 105–129.
- Bauer AM, Lamb T, Branch WR. 2006. A revision of the *Pachydactylus serval* and *P. weberi* groups (Reptilia: Gekkota: Gekkonidae) of Southern Africa, and with the description of eight new species. *Proceedings of the California Academy of Sciences Series 4* 57(23): 595–709.
- Berg MP van den. 2017. An annotated bibliographic history of *Ichnotropis* Peters, 1854 (Reptilia, Lacertidae) with remarks on the validity of some of the including species. *L@CERTIDAE (Eidechsen Online)* 2017(4): 60–138.
- BirdLife International. 2018. *Important Bird Areas Factsheet: Bicular National Park*. Available: <http://www.birdlife.org> [Accessed: 27 April 2018].
- Bittencourt-Silva GB. 2019. A herpetological survey of western Zambia. *Amphibian & Reptile Conservation* 13(2) [Special Section]: 1–28 (e181).
- Bocage JVB. 1873. Melanges erpetologiques. II. Sur quelques reptiles et batraciens nouveaux, rares ou peu connus d'Afrique occidentale. *Jornal de Sciencias Mathematicas, Physicas e Naturaes* (Lisboa) 4: 209–227.
- Bocage JVB. 1879. Reptiles et batraciens nouveaux d'Angola. *Jornal de Sciencias, Mathematicas, Physicas e Naturaes* (Lisboa) 7(26): 87–99.
- Bocage JVB. 1887. Sur un *Python* nouveau d'Afrique. *Jornal de Sciencias, Mathematicas, Physicas e Naturaes* (Lisboa) 12: 87–88.
- Bocage JVB. 1895. *Herpétologie d'Angola et du Congo*. Imprimerie Nationale, Lisbonne, Portugal. 203 p.
- Bocage JVB. 1897. Mammiferos, Aves e reptis da Hanha, no Sertão de Benguella. *Jornal de Sciencias, Mathematicas, Physicas e Naturaes* (Lisboa) Series 2, 4: 207–211.
- Bogert CM. 1940. Herpetological results of the Vernay Angola Expedition with notes on African reptiles in other collections. I. Snakes, including an arrangement of African Colubridae. *Bulletin of the American Museum of Natural History* 77: 1–107.
- Boulenger GA. 1905. A list of the batrachians and reptiles collected by Dr. W.J. Ansorge in Angola, with descriptions of new species. *Annals and Magazine of Natural History Series 7*, 16: 8–115.

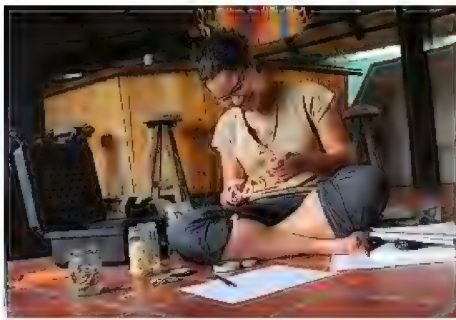
- Boulenger GA. 1907. Description of a new frog discovered by Dr. W.J. Ansorge in Mossamedes, Angola. *Annals and Magazine of Natural History Series 7*, 20: 109.
- Boulenger GA. 1919. On *Rana ornatissima*, Bocage, and *R. ruddi*, Blgr. *Transactions of the Royal Society of South Africa* 8: 33–37.
- Branch B. 2012. *Tortoises, Terrapins, and Turtles of Africa*. Random House Struik, Cape Town, South Africa. 128 p.
- Branch WR. 1998. *Field Guide to Snakes and other Reptiles of Southern Africa*. Struik, Cape Town, South Africa. 399 p.
- Branch WR. 2018. Snakes of Angola: an annotated checklist. *Amphibian & Reptile Conservation* 12(2) [General Section]: 41–82 (e159).
- Branch WR, Baptista N, Vaz Pinto P. 2018. Angolan amphisbaenians: rediscovery of *Monopeltis luandae* Gans 1976, with comments on the type locality of *Monopeltis perplexus* Gans 1976 (Sauria: Amphisbaenidae). *Herpetology Notes* 11: 603–606.
- Branch WR, Baptista N, Keates C, Edwards S. 2019a. Rediscovery, taxonomic status, and phylogenetic relationships of two rare and endemic snakes (Serpentes: Psammophinae) from the southwestern Angolan plateau. *Zootaxa* 4590(3): 342–366.
- Branch WR, Conradie W, Vaz Pinto P, Tolley K. 2019b. Another Angolan Namib endemic species: a new *Nucras* Gray, 1838 (Squamata: Lacertidae) from south-western Angola. *Amphibian & Reptile Conservation* 13(2) [Special Section]: 82–95 (e199).
- Branch WR, Haacke W, Vaz Pinto P, Conradie W, Baptista N, Verburgt L. 2017. Loveridge's Angolan geckos, *Afroedura karroica bogerti* and *Pachydactylus scutatus angolensis* (Sauria, Gekkonidae): new distribution records, comments on type localities, and taxonomic status. *Zoosystematics and Evolution* 93(1): 157–166.
- Branch WR, McCartney CJ. 1992. A report on a small collection of reptiles from southern Angola. *Journal of the Herpetological Association of Africa* 41: 1–3.
- Branch WR, Vaz Pinto P, Baptista N, Conradie W. 2019c. The reptiles of Angola – history, updated checklists, endemism, hot spots, and future directions for research. Pp. 283–344 In: *Biodiversity of Angola. Science and Conservation: A Modern Synthesis*. Editors, Huntley BJ, Russo V, Lages F, Ferrand N. Springer, Cham, Switzerland. 549 p.
- Broadley DG. 1971. A revision of the African snake genera *Amblyodipsas* and *Xenocalamus*. *Occasional Papers of the National Museums of Rhodesia, Series B, Natural Sciences* 4(33): 629–697.
- Broadley DG. 1995. The Snouted Cobra, *Naja annulifera*, a valid species in southern Africa. *Journal of the Herpetological Association of Africa* 44(2): 26–33.
- Broadley DG. 1997. A review of the *Monopeltis capensis* complex in southern Africa (Reptilia: Amphisbaeniidae). *African Journal of Herpetology* 46(1): 1–12.
- Broadley DG. 2002. A review of the species of *Psammophis* Boie found south of latitude 12° S (Serpentes: Psammophiinae). *African Journal of Herpetology* 51(2): 83–120.
- Broadley DG, Broadley S. 1997. A revision of the African genus *Zygaspis* Cope (Reptilia: Amphisbaenia). *Syntarsus* 4: 1–24.
- Broadley DG, Broadley S. 1999. A review of the African worm snakes from south of latitude 12°S (Serpentes: Leptotyphlopidae). *Syntarsus* 5: 1–36.
- Broadley DG, Gans C, Visser J. 1976. Studies on amphisbaenians (Amphisbaenia, Reptilia). 6. The genera *Monopeltis* and *Dalophia* in southern Africa. *Bulletin of the American Museum of Natural History* 157(5): 311–486.
- Broadley DG, Wallach V. 2009. A review of the eastern and southern African blind-snakes (Serpentes: Typhlopidae), excluding *Letheobia* Cope, with the description of two new genera and a new species. *Zootaxa* 2255: 1–100.
- Burgess N, Hales JD, Underwood E, Dinerstein E, Olson D, Itoua I, Schipper J, Ricketts T, Newman K. 2004. *Terrestrial Ecoregions of Africa and Madagascar – a Conservation Assessment*. Island Press, Washington DC, USA. 499 p.
- Butler BO, Ceriaco LPM, Marques MP, Bandeira S, Júlio T, Heinicke MP, Bauer AM. 2019. Herpetological survey of Huíla Province, southwest Angola, including first records from Bicular National Park. *Herpetological Review* 50(2): 225–240.
- Ceriaco LMP, Bauer AM, Blackburn DC, Lavres ACFC. 2014. The herpetofauna of the Capanda Dam region, Malanje, Angola. *Herpetological Review* 45(4): 667–674.
- Ceriaco LMP, Branch WR, Bauer AM. 2018a. A new species of African snake-eyed skink (Scincidae: *Panaspis*) from central and northern Namibia. *Zootaxa* 4527: 140–150.
- Ceriaco LMP, Marques MP, Bandeira S, Agarwal I, Stanley EL, Bauer AM, Heinicke MP, Blackburn DC. 2018b. A new earless species of *Poyntonophrynus* (Anura, Bufonidae) from the Serra da Neve Inselberg, Namibe Province, Angola. *ZooKeys* 780: 109–136.
- Ceriaco LMP, Marques MP, Bandeira S, Blackburn DC, Bauer AM. 2018c. Herpetological survey of Cangandala National Park, with a synoptic list of the amphibians and reptiles of Malanje Province, Central Angola. *Herpetological Review* 49(3): 408–431.
- Ceriaco LMP, de Sá SC, Bandeira S, Valério H, Stanley EL, Kuhn AL, Marques M, Vindum JV, Blackburn DC, Bauer AM. 2016. Herpetological survey of Iona National Park and Namibe Regional Natural Park, with a synoptic list of the amphibians and reptiles of Namibe Province, southwestern Angola. *Proceedings of the California Academy of Sciences* 63(2): 15–61.

- Channing A. 2001. *Amphibians of Central and Southern Africa*. Cornell University Press, Ithaca, New York, USA. 470 p.
- Channing A, Becker F. 2019. Correction to the type locality of *Tomopterna ahli* (Deckert, 1938) (Anura: Pyxicephalidae), with the designation of a neotype. *Zootaxa* 4688(4): 549–560.
- Channing A, Bogart JP. 1996. Description of a tetraploid *Tomopterna* (Anura: Ranidae) from South Africa. *South African Journal of Zoology* 31: 80–85.
- Channing A, Hillers A, Lötters S, Rödel MO, Schick S, Conradie W, Rödder D, Mercurio V, Wagner P, Dehling JM, et al. 2013. Taxonomy of the super-cryptic *Hyperolius nasutus* group of long reed frogs of Africa (Anura: Hyperoliidae), with descriptions of six new species. *Zootaxa* 3620: 301–350.
- Channing A, Rödel MO, Channing J. 2012. *Tadpoles of Africa: The Biology and Identification of All Known Tadpoles in sub-Saharan Africa*. Edition Chimaira, Frankfurt, Germany. 402 p.
- Channing A, Rödel MO. 2019. *Field Guide to the Frogs and Other Amphibians of Africa*. Struik Nature, Cape Town, South Africa. 408 p.
- Chisingui AV, Gonçalves FMP, Tchamba JJ, Camôngua LJ, Rafael MFF, Alexandre JLM. 2018. Vegetation survey of the woodlands of Huíla Province. *Biodiversity & Ecology* 6: 426–437.
- Conradie W, Bills R, Branch WR. 2016. The herpetofauna of the Cubango, Cuito, and lower Cuando river catchments of south-eastern Angola. *Amphibian & Reptile Conservation* 10(2) [Special Section]: 6–36 (e126).
- Conradie W, Branch WR, Measey GJ, Tolley KA. 2012. A new species of *Hyperolius* Rapp, 1842 (Anura: Hyperoliidae) from the Serra da Chela mountains, south-western Angola. *Zootaxa* 3269: 1–17.
- Conradie W, Branch WR, Tolley KA. 2013. Fifty Shades of Grey: giving color to the poorly known Angolan Ashy Reed Frog (Hyperoliidae: *Hyperolius cinereus*), with the description of a new species. *Zootaxa* 3635(3): 201–223.
- Conradie WC, Bills R, Baptista N, Verburgt L, Harvey J, Branch WR. 2017. Oral presentation. Across river basins: exploring the unknown southeastern Angola [abstract]. *African Herp News* 66: 14–15.
- Conroy CJ, Papenfuss T, Parker J, Hahn NE. 2009. Use of tricaine methanesulfonate (MS222) for euthanasia of reptiles. *Journal of the American Association for Laboratory Animal Science* 48(1): 28–32.
- Crawford-Cabral J, Mesquitela LM. 1989. *Índice Toponímico de Colheitas Zoológicas em Angola (Mammalia, Aves, Reptilia e Amphibia)*. Instituto de Investigação Científica Tropical, Lisboa, Portugal. 206 p.
- Dean WRJ. 2000. *The Birds of Angola: an Annotated Checklist*. BOU Checklist Series 18. British Ornithologist's Union, Tring, United Kingdom. 433 p.
- Diniz AC. 2006. *Características Mesológicas de Angola*. Instituto Português de Apoio ao Desenvolvimento, Lisboa, Portugal. 546 p.
- du Preez L, Carruthers V. 2009. *A Complete Guide to the Frogs of Southern Africa*. Struik Publishers, Cape Town, South Africa. 488 p.
- du Preez L, Carruthers V. 2017. *Frogs of Southern Africa – A Complete Guide*. Struik Publishers, Cape Town, South Africa. 520 p.
- Edwards S, Branch WR, Vanhooydonck B, Herrel A, Measey JG, Tolley KA. 2013. Taxonomic adjustments in the systematics of the southern African lacertid lizards (Sauria: Lacertidae). *Zootaxa* 3669(2): 101–114.
- Eimermacher TG. 2012. Phylogenetic systematics of Dispholidine colubrids (Serpentes: Colubridae). Ph.D. Dissertation, University of Texas at Arlington, Arlington, Texas, USA.
- Engelbrecht HM, Branch WR, Greenbaum E, Alexander GJ, Jackson K, Burger M, Conradie W, Kusamba C, Zassi-Boulou A-G, Tolley KA. 2019. Diversifying into the branches: species boundaries in African green and bush snakes, *Philothamnus* (Serpentes: Colubridae). *Molecular Phylogenetics and Evolution* 130: 357–365.
- Ferreira JB. 1897. Lista dos reptis e amphibios que fazem parte da última remessa de J. d'Anchieta. *Jornal de Ciências Matemáticas, Physicas e Naturaes* Segunda série 5: 240–246.
- Ferreira JB. 1902. Reptis de Angola da região ao norte do Quanza da collecção Pereira do Nascimento. *Jornal de Ciências Matemáticas, Physicas e Naturaes* Segunda série 7: 9–16.
- Ferreira JB. 1904. Reptis e amphibios de Angola da região ao norte do Quanza (Collecção Newton – 1903). *Jornal de Ciências Matemáticas, Physicas e Naturaes* Segunda série 7: 111–117.
- FitzSimons VF. 1941. Descriptions of some new lizards from South Africa and a frog from Southern Rhodesia. *Annals of the Transvaal Museum* 20(3): 273–281.
- Frost DR. 2019. Amphibian Species of the World: an online reference. Version 6.0. American Museum of Natural History, New York, New York, USA. Available: <http://research.amnh.org/vz/herpetology/amphibia/index.php> [Accessed: 20 February 2019].
- Furman BL, Bewick AJ, Harrison TL, Greenbaum E, Gvoždík V, Kusamba C, Evans BJ. 2015. Pan-African phylogeography of a model organism, the African clawed frog *Xenopus laevis*. *Molecular Ecology* 24(4): 909–925.
- Gans C. 1976. Three new spade-snouted amphisbaenians from Angola (Amphisbaenia, Reptilia). *American Museum Novitates* 2590: 1–11.
- Haacke WD. 1984. The herpetology of the southern Kalahari domain. *Koedoe* 27(2): 171–186.
- Haacke WD. 1997. Systematics and biogeography of

- the southern African scincine genus *Typhlacontias* (Reptilia: Scincidae). *Bonner Zoologische Beiträge* 47: 139–164.
- Haacke WD. 2008. A new leaf-toed gecko (Reptilia: Gekkonidae) from south-western Angola. *African Journal of Herpetology* 57: 85–92.
- Heinicke MP, Jackman TR, Bauer AM. 2017. The measure of success: geographic isolation promotes diversification in *Pachydactylus* geckos. *BMC Evolutionary Biology* 17: 9.
- Hellmich W. 1957. Herpetologische Ergebnisse einer Forschungsreise in Angola. *Veröffentlichungen der Zoologischen Staatssammlung München* 5: 1–92.
- Herrmann HW, Branch WR. 2013. Fifty years of herpetological research in the Namib Desert and Namibia with an updated and annotated species checklist. *Journal of Arid Environments* 93: 94–115.
- Huntley BJ. 1974. Outlines of wildlife conservation in Angola. *South African Journal of Wildlife* 4: 157–166.
- Huntley BJ. 2009. *SANBI/ISCED/UAN Angolan Biodiversity Assessment Capacity Building Project - Report on Pilot Project*. Unpublished report. 111 p.
- Huntley BJ. 2019. Angola in outline: physiography, climate and patterns of biodiversity. Pp. 15–42 In: *Biodiversity of Angola. Science & Conservation: A Modern Synthesis*. Editors, Huntley BJ, Russo V, Lages F, Ferrand N. Springer, Cham, Switzerland. 549 p.
- Huntley BJ, Beja P, Vaz Pinto P, Russo V, Veríssimo L, Morais M. 2019a. Biodiversity conservation: history, protected areas and hotspots. Pp. 495–512 In: *Biodiversity of Angola. Science & Conservation: A Modern Synthesis*. Editors, Huntley BJ, Russo V, Lages F, Ferrand N. Springer, Cham, Switzerland. 549 p.
- Huntley BJ, Russo V, Lages F, Ferrand N (Editors). 2019b. *Biodiversity of Angola. Science & Conservation: A Modern Synthesis*. Springer, Cham, Switzerland. 549 p.
- Jones A, Breuning-Madsen H, Brossard M, Dampha A, Deckers J, Dewitte O, Gallali T, Hallett S, Jones R, Kilasara M, et al. 2013. *Soil Atlas of Africa*. Publications Office of the European Union, Brussels, Belgium. 176 p.
- Jürgens N, Strohbach B, Lages F, Schmiedel U, Finckh M, Sichone P, Hahn L-M, Ziegelski P. 2018. Biodiversity observation – an overview of the current state and first results of biodiversity monitoring studies. *Biodiversity & Ecology* 6: 382–396.
- Kelly CMR, Barker NP, Villet MH, Broadley DG, Branch WR. 2008. The snake family Psammophiidae (Reptilia: Serpentes): phylogenetics and species delimitation in the African sand snakes (*Psammophis* Boie, 1825) and allied genera. *Molecular Phylogenetics and Evolution* 47(3): 1,045–1,060.
- Kindler C, Branch WR, Hofmeyr MD, Maran J, Šíroký P, Vences M, Harvey J, Hauswaldt S, Schleicher A, Stuckas H, et al. 2012. Molecular phylogeny of African hinge-back tortoises (*Kinixys*): implications for phylogeography and taxonomy (Testudines: Testudinidae). *Journal of Zoological Systematics and Evolutionary Research* 50(3): 192–201.
- Laurent RF. 1950. Reptiles et batraciens de la region de Dundo (Angola du Nord-Est). *Publicações Culturais da Companhia de Diamantes de Angola* 10: 7–17.
- Laurent RF. 1954. Reptiles et batraciens de la région de Dundo (Angola) (Deuxième note). *Publicações Culturais da Companhia de Diamantes de Angola* 23: 35–84.
- Laurent RF. 1957. Notes sur les Hyperoliidae. *Revue de Zoologie et de Botanique Africaines* 56: 274–282.
- Laurent RF. 1964. Reptiles et amphibiens de l'Angola (Troisième contribution). *Publicações Culturais da Companhia de Diamantes de Angola* 67: 1–165.
- Leaché AD, Wagner P, Linkem CW, Böhme W, Papenfuss TJ, Chong RA, Lavin BR, Bauer AM, Nielsen SV, Greenbaum E, et al. 2014. A hybrid phylogenetic–phylogenomic approach for species tree estimation in African *Agama* lizards with applications to biogeography, character evolution, and diversification. *Molecular Phylogenetics and Evolution* 79: 215–230.
- Leistner OA. 1967. The plant ecology of the southern Kalahari. *Memoirs of the Botanical Survey of South Africa* 38: 1–172.
- Loveridge A. 1941. Revision of the African lizards of the family Amphisbaenidae. *Bulletin of the Museum of Comparative Zoology* 87(5): 353–451.
- Manaças S. 1963. Saurios de Angola. *Memórias da Junta de Investigação Científica do Ultramar* 43(2): 223–240.
- Manaças S. 1981. Ofídeos venenosos da Guiné, S. Tomé, Angola e Moçambique. *Garcia de Orta: Série de Zoologia* 10(1/2): 13–46.
- Marais J. 2004. *A Complete Guide to the Snakes of Southern Africa*. Struik, Cape Town, South Africa. 312 p.
- Marques MP, Ceríaco LMP, Blackburn DC, Bauer AM. 2018. Diversity and distribution of the amphibians and terrestrial reptiles of Angola: atlas of historical and bibliographic records (1840–2017). *Proceedings of the California Academy of Sciences* 65(Supplement II): 1–501.
- Medina MF, Bauer AM, Branch WR, Schmitz A, Conradie W, Nagy ZT, Hibbits TJ, Ernst R, Portik DM, Nielsen SV, et al. 2016. Molecular phylogeny of *Panaspis* and *Afroablepharus* skinks (Squamata: Scincidae) in the savannas of sub-Saharan Africa. *Molecular Phylogenetics and Evolution* 100: 409–423.
- Mendelsohn JM. 2019. Landscape changes in Angola. Pp. 123–137 In: *Biodiversity of Angola. Science & Conservation: a Modern Synthesis*. Editors, Huntley BJ, Russo V, Lages F, Ferrand N. Springer, Cham,

- Switzerland. 549 p.
- Mendelsohn JM, Mendelsohn S. 2018. *Sudoeste de Angola: um Retrato da Terra e da Vida, South West Angola: a Portrait of Land and Life*. Arte & Ciência, Porto, Portugal. 424 p.
- Mertens R. 1938. Amphibien und Reptilien aus Angola gesammelt von W. Schack. *Senckenbergiana* 20: 425–443.
- Missão de Pedologia de Angola. 1959. Carta geral dos solos de Angola. I - Distrito da Huila. *Memórias da Junta de Investigações do Ultramar* 9: 1–482.
- Monard A. 1931. Mission scientifique Suisse dans l'Angola. Résultats scientifiques. Reptiles. *Bulletin de la Société Neuchâtel Sciences Naturelles* 55: 89–111.
- Monard A. 1937a. Contribution à la batrachologie d'Angola. *Bulletin de la Société Neuchâteloise des Sciences Naturelles* 62: 1–59.
- Monard A. 1937b. Contribution à l'herpétologie d'Angola. *Arquivos do Museu Bocage* 8: 19–154.
- Overton J, Fernandes S, Elizalde D, Groom R, Funston P. 2017. *A Large Mammal Survey of Bicular and Mupa National Parks, Angola*. Unpublished report. Instituto Nacional da Biodiversidade e Áreas de Conservação, Luanda, Angola.
- Perret J-L. 1976. Révision des amphibiens africains et principalement des types, conservés au Musée Bocage de Lisbonne. *Arquivos do Museu Bocage Series 2*, 6: 15–34.
- Petzold A, Vargas-Ramírez M, Kehlmaier C, Vamberger M, Branch WR, du Preez L, Hofmeyr MD, Meyer L, Schleicher A, Široký P, et al. 2014. Diagnoses for African helmeted terrapins (Testudines: Pelomedusidae: *Pelomedusa*), with the description of six new species. *Zootaxa* 3795(5): 523–548.
- Pietersen DW, Pietersen EW, Conradie W. 2017. Preliminary herpetological survey of Ngonye Falls and surrounding regions in south-western Zambia. *Amphibian & Reptile Conservation* 11(1): 24–43 (e148).
- Portillo F, Branch WR, Conradie W, Rödel MO, Penner J, Barej MF, Kusamba C, Muninga WM, Aristote MM, Bauer AM, et al. 2018. Phylogeny and biogeography of the African burrowing snake subfamily Aparallactinae (Squamata: Lamprophiidae). *Molecular Phylogenetics and Evolution* 127: 288–303.
- Poynton JC, Haacke WD. 1993. On a collection of amphibians from Angola, including a new species of *Bufo* Laurenti. *Annals of the Transvaal Museum* 36: 9–16.
- Poynton JC, Loader SP, Conradie W, Rödel MO, Liedtke HC. 2016. Designation and description of a neotype of *Sclerophrys maculata* (Hallowell, 1854), and reinstatement of *S. pusilla* (Mertens, 1937) (Amphibia: Anura: Bufonidae). *Zootaxa* 4098: 73–94.
- Rasmussen J. 2005. On the identification and distribution of the two-striped night adder (*Causus bilineatus*) and related forms. *African Journal of Herpetology* 54(1): 1–15.
- Roll U, Feldman A, Novosolov M, Allison A, Bauer AM, Bernard R, Böhm M, Castro-Herrera F, Chirio L, Collen B, et al. 2017. The global distribution of tetrapods reveals a need for targeted reptile conservation. *Nature Ecology & Conservation* 1: 1,677–1,682.
- Ruas C. 1996. Contribuição para o conhecimento da fauna de batráquios de Angola. *Garcia de Orta: Série de Zoologia* 21(1): 19–41.
- Ruas C. 2002. Batráquios de Angola em colecção no Centro de Zoologia. *Garcia de Orta: Série de Zoologia* 24(1–2): 139–154.
- Russo V, Huntley BJ, Lages F, Ferrand N. 2019. Conclusions: biodiversity research and conservation opportunities. Pp. 543–549 In: *Biodiversity of Angola. Science & Conservation: a Modern Synthesis*. Editors, Huntley BJ, Russo V, Lages F, Ferrand N. Springer, Cham, Switzerland. 549 p.
- SASSCAL. 2018. ObservationNet. Available: <http://www.sasscalobservationnet.org/> [Accessed 12 August 2018].
- SASSCAL. 2019. WeatherNet. Available: <http://www.sasscalweather.net.org/> [Accessed 12 September 2019].
- Schiøtz A. 1999. *Treefrogs of Africa*. Edition Chimaira, Frankfurt, Germany. 350 p.
- Schmidt KP. 1933. The reptiles of the Pulitzer Angola expedition. *Annals of the Carnegie Museum* 22: 1–15.
- Schmidt KP. 1936. The amphibians of the Pulitzer-Angola Expedition. *Annals of the Carnegie Museum* 25: 127–133.
- Schmidt KP, Inger RF. 1959. Amphibians, exclusive of the genera *Afraxalus* and *Hyperolius*. *Exploration du Parc National de l'Upemba. Miss. G.F. de Witte, en collaboration avec W. Adam, A. Janssens, L. van Meel et R. Verheyen (1946–1949)*. 56: 1–264.
- Scholes RJ, Frost PG, Tian Y. 2004. Canopy structure in savannas along a moisture gradient on Kalahari sands. *Global Change Biology* 10(3): 292–302.
- Sigovini M, Keppel E, Tagliapietra D. 2016. Open nomenclature in the biodiversity era. *Methods in Ecology and Evolution* 7(10): 1,217–1,225.
- Simões AP. 1971. *Considerações acerca de Algumas Zonas de Protecção da Fauna, Flora e Solo de Angola*. Instituto de Investigação Científica de Angola, Luanda, Angola. 78 p.
- Stanley EL, Ceríaco LMP, Bandeira S, Valerio H, Bates MF, Branch WR. 2016. A review of *Cordylus machadoi* (Squamata: Cordylidae) in southwestern Angola, with the description of a new species from the Pro-Namib desert. *Zootaxa* 4061(3): 201–226.
- Tandy M, Keith R. 1972. *Bufo* of Africa. Pp. 119–170 in *Evolution in the Genus Bufo*. Editor, Blair WF. University of Texas Press, Austin, Texas, USA. 459 p.
- Teixeira JB. 1968. *Parque Nacional do Bicular. Carta da Vegetação (1ª aproximação) e Memória*

- Descritiva*. Instituto de Investigação Agronómica de Angola, Nova Lisboa, Angola. 43 p.
- Thys van den Audenaerde DFE. 1967. Les serpents des environs de Dundo (Angola). Note complémentaire. *Publicações Culturais da Companhia de Diamantes de Angola* 76: 31–37.
- Trape JF, Crochet PA, Broadley DG, Sourouille P, Mané Y, Burger M, Böhme W, Saleh M, Karan A, Lanza B, et al. 2019. On the *Psammophis sibilans* group (Serpentes, Lamprophiidae, Psammophiinae) north of 12°S, with the description of a new species from West Africa. *Bonn Zoological Bulletin* 68(1): 61–91.
- Turtle Taxonomy Working Group [Rhodin AGJ, Iverson JB, Fritz BR, Georges A, Shaffer HB, van Dijk PP.] 2017. Turtles of the world: Annotated checklist and atlas of taxonomy, synonymy, distribution, and conservation status (8th edition). In: *Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group*. Editors, Rhodin AGJ, Iverson JB, van Dijk PP, Saumure RA, Buhlmann KA, Pritchard PCH, Mittermeier RA. *Chelonian Research Monographs* 7. IUCN, Gland, Switzerland. 292 p.
- Uetz P, Freed P, Hošek P. 2019. The Reptile Database. Available: <http://www.reptile-database.org> [Accessed: 15 February 2019].
- Vaz Pinto P, Verissimo L, Branch WR. 2019. Hiding in the bushes for 110 years: rediscovery of an iconic Angolan gecko (*Afrogecko ansorgii* Boulenger, 1907, Sauria: Gekkonidae). *Amphibian & Reptile Conservation* 13(2) [Special Section]: 29–41 (e182).
- Wagner P, Greenbaum E, Bauer AM, Kusamba C, Leaché AD. 2018. Lifting the blue-headed veil – integrative taxonomy of the *Acanthocercus atricollis* species complex (Squamata: Agamidae). *Journal of Natural History* 52: 771–817.
- Wilson L, Channing A. 2019. A new sand frog from Namaqualand, South Africa (Pyxicephalidae: *Tomopterna*). *Zootaxa* 4609(2): 225–246.
- Wüster W, Crookes S, Ineich I, Mané Y, Pook CE, Trape J-F, Broadley DG. 2007. The phylogeny of cobras from mitochondrial DNA sequences, evolution of venom spitting, and the phylogeography of the African spitting cobras (Serpentes, Elapidae, *Naja nigricollis* complex). *Molecular Phylogenetics and Evolution* 45(2): 437–453.
- Zimkus BM, Rödel MO, Hillers A. 2010. Complex patterns of continental speciation: molecular phylogenetics and biogeography of sub-Saharan puddle frogs (*Phrynobatrachus*). *Molecular Phylogenetics and Evolution* 55(3): 883–900.



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Telmo António is an Angolan biologist who recently graduated in Biology Teaching from ISCED-Huíla, with an honors thesis on the grasses of Tundavala. Telmo worked as research intern in the SASSCAL project at ISCED-Huíla from 2016 to 2018, participating in all tasks related to plants, mammals, and herpetofauna, especially in Tundavala and Bicular National Park. Telmo taught high-school Biology in Lubango, Angola. He is currently a Natural Resources Management M.Sc. student in the Namibia University of Science and Technology, within the SCIONA Project “Co-designing conservation technologies for Iona - Skeleton Coast Transfrontier Conservation Area (Angola - Namibia),” which is funded by the European Union.



Bill Branch (1946–2018) was born in London and worked as Curator of Herpetology at the Port Elizabeth Museum for over 30 years (1979–2011), and upon his retirement he was appointed as Curator Emeritus of Herpetology. His herpetological studies have focused mainly on the systematics, phylogenetic relationships, and conservation of African reptiles. He has published over 300 scientific papers, and numerous popular articles and books. The latter include: *South African Red Data Book of Reptiles and Amphibians* (1988), *Dangerous Snakes of Africa* (1995, with Steve Spawls), *Field Guide to the Reptiles of Southern Africa* (1998), *Tortoises, Terrapins and Turtles of Africa* (2008), and *Atlas and Red Data Book of the Reptiles of South Africa, Lesotho and Swaziland* (multi-authored, 2014), as well as smaller photographic guides. In 2004 Bill was the 4th recipient of the “Exceptional Contribution to Herpetology” award of the Herpetological Association of Africa. Bill has undertaken field work in over 16 African countries, and described nearly 50 species, including geckos, lacertids, chameleons, cordylids, tortoises, adders, and frogs. He supervised all tasks related to herpetology in the SASSCAL project, the creation of a herpetofauna archive in ISCED-Huíla, and many other ongoing initiatives in Angolan herpetology.

Appendix 1. List of collecting sites in Bicular National Park, Angola, and surroundings, with coordinates (decimal degrees). Asterisks (*) indicate sites located outside of the park. Sampling method: AS = active searching or opportunistic observation; DOR = dead on road; T = trapping.

Site	Name	Latitude (°S)	Longitude (°E)	Date or date range	Sampling method	Habitat type
1	BNP, road between Tunda Gate and Main Camp	-15.610300	14.879561	6 Nov 2017	DOR	Grassland along Luconda drainage line
2	BNP, near Tchiwacussi	-15.189030	15.254460	6 Nov 2017	AS	Secondary growth scrubland in old crop fields, near Tambi drainage line
3	BNP	-15.177040	15.228570	6 Nov 2017	AS	Degraded scrubland near Tambi drainage line
5	BNP	-15.148550	14.841380	16 Mar 2018	AS	Miombo woodland with considerable bush encroachment
6	BNP	-15.130452	14.683723	6 Dec 2016	AS	Recently burnt open miombo woodland
7	BNP	-15.129961	14.730298	6 Dec 2016	AS	Temporary pond along Bicular drainage line
9	BNP	-15.126656	14.637726	6 Dec 2016	AS	Recently burnt open miombo woodland
10	BNP	-15.126032	14.601210	6 Dec 2016	AS	Open miombo woodland
12	BNP, road along Bicular drainage line	-15.104853	14.840320	7 Nov 2017	AS	Grassland
13	BNP	-15.100510	14.845050	6 Dec 2016	AS	Open miombo woodland
14	BNP, close to Main Camp, road to Hombo gate	-15.096715	14.839058	2 Dec 2016	AS	Leaf litter accumulated along the road, in open miombo woodland
15	BNP, drainage line upstream Main Camp water hole	-15.092432	14.836883	2 Dec 2016	AS	Grassland
16	BNP, road between Capelongo Gate and Lagoa da Matamba	-15.082880	14.928760	2 Dec 2016	AS	Dense miombo woodland
17	BNP, road between Capelongo Gate and Main Camp	-15.057374	14.932907	7 Dec 2017	AS	Dense miombo woodland
18	BNP, road between Capelongo Gate and Main Camp	-15.036282	14.959543	7 Dec 2017	AS	Mosaic of dense miombo woodland and shrubland
19	BNP	-15.160722	14.859036	7 Dec 2017	AS	Miombo woodland
20	BNP	-15.129161	14.881776	2 Jul 2017	AS	Open miombo woodland
21*	Outskirts of BNP	-14.945094	15.095214	6 Nov 2017	DOR	Degraded open shrubland
25	Lagoa da Matamba	-15.122320	14.902980	4 Nov 2017	AS	Permanent water body with aquatic vegetation
26	Lagoa do Djimbi	-15.145520	14.914670	5 Nov 2017	AS	Permanent water body
27	Main Camp water whole	-15.102406	14.836857	8 Dec 2016	AS	Permanent water body
28	Main Camp water whole	-15.098320	14.837030	7 Dec 2016	AS	Permanent water body
29	Main Camp water whole	-15.098060	14.836990	7 Dec 2016	AS	Permanent water body
30	Drainage line upstream Main Camp water whole	-15.093490	14.837450	4 Dec 2016	AS	Grassland
31	BNP Main Camp	-15.100725	14.839557	2009–2017	AS	Human facilities, sandy soils, tall <i>Burkea africana</i> trees, surrounded miombo woodland
32*	Outskirts of BNP	-15.060080	15.251800	6 Nov 2017	AS	Degraded open shrubland
33	BNP	-15.150060	14.812300	5 Nov 2017	AS	Miombo woodland with considerable bush encroachment
34	BNP, near Bicular drainage line	-15.243460	14.891460	5 Nov 2017	AS	Recently burnt open miombo woodland
35	BNP, road between Hombo Gate and Main Camp	-15.024986	14.796841	4 Nov 2017	AS	Open miombo woodland with regenerating understorey

Herpetofauna of Bicular National Park, Angola

Appendix 1. List of collecting sites in Bicular National Park, Angola, and surroundings, with coordinates (decimal degrees). Asterisks (*) indicate sites located outside of the park. Sampling method: AS = active searching or opportunistic observation; DOR = dead on road; T = trapping.

Site	Name	Latitude (°S)	Longitude (°E)	Date or date range	Sampling method	Habitat type
36	BNP, close to Main Camp, road to Hombo gate	-15.095409	14.838751	2 Nov 2016	AS	Miombo woodland
39	BNP, Tunda Gate	-15.645000	14.708333	6 Dec 2015	AS	Dry woodland on deep sands
40	BNP	-15.133300	14.900000	11 Dec 2015	AS	Ecotone between grassland and woodland
41*	Lagoa Nougafafa, outskirts of BNP	-14.977117	14.693638	6 Dec 2016	AS	Permanent water body
42*	Between Chibemba and Cahama	-16.03	14.20	15 Feb 2010	AS	Small wetland, approximate coordinates
43	BNP	-15.042928	14.805010	5 Dec 2016	AS	Border of one a grassland valley, in the ecotone between suffrutex grasslands and woodland, crossing sandy dirt road
CF*	Carmira Farm	-16.044722	14.569167	2015–2018	AS	Baikiaea/Burkea woodland, deep Kalahari sands
C1*	Road to Carmira Farm	-15.992383	14.410287	5 Apr 2018	AS	Baikiaea/Burkea woodland, deep Kalahari sands
HF*	Handa Farm	-14.699722	14.295833	2015–2018	AS	Degraded miombo woodland with overgrazing and deforestation
T1	Woodland trapline 1	-15.094405	14.838312	2–7 Dec 2016	T	Miombo woodland not burnt for more than 1 year
T2	Woodland trapline 2	-15.100358	14.838958	7–10 Dec 2016	T	Miombo woodland not burnt for more than 5 years
T3	Grassland trapline	-15.102190	14.837057	2–10 Dec 2016	T	Grassland near permanent body of water



First record of the Cat Ba Tiger Gecko, *Goniurosaurus catbaensis*, from Ha Long Bay, Quang Ninh Province, Vietnam: microhabitat selection, potential distribution, and evidence of threats

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Abstract.—The Cat Ba Tiger Gecko (*Goniurosaurus catbaensis*) was described from Cat Ba Island, Hai Phong, northern Vietnam in 2008, while a presumed congener was recently spotted from another offshore island in the Ha Long Bay. During the field surveys reported here, new *Goniurosaurus* occurrences were discovered for the first time on small offshore islands in the Ha Long Bay, Quang Ninh Province. These were identified and confirmed as *G. catbaensis* based on morphological and molecular data. However, these newly found populations are very small and exposed to increasing anthropogenic pressures. Since knowledge about the species ecology remains poor, the first microhabitat characterization for *G. catbaensis* is provided herein, which is essential for conservation of the species as well as its natural habitats. Sex- and age-related differences in selection of perch height are herein presented. In addition, we present evidence for various anthropogenic threats such as regular trade in living tiger geckos (including *G. catbaensis*) on local markets in Hai Phong and Ho Chi Minh cities, Vietnam. These findings highlight the need for more stringent conservation measures to reduce human impacts on the extremely small, insular populations of the Cat Ba Tiger Gecko.

Key words. Anthropogenic pressure, conservation, ecology, offshore islands, phylogram, trade

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Introduction

The genus *Goniurosaurus* currently comprises 19 species with a disjunct distribution in southern East Asia. Most *Goniurosaurus* species are endemic with restricted distribution ranges (Chen et al. 2014; Grismer et al. 1994, 1999; Honda and Ota 2017; Seufer et al. 2005; Yang and Chan 2015; Zhou et al. 2018; Ziegler et al. 2008). Habitat degradation and overharvesting for the pet trade were identified as major threats to wild populations of tiger geckos (Yang and Chan 2015). At present, five species of *Goniurosaurus* are known from Vietnam, namely *G.*

araneus, *G. catbaensis*, *G. huiliensis*, *G. lichtenfelderi*, and *G. luii* (Nguyen et al. 2009). Among these species, the insular Cat Ba Tiger Gecko (*Goniurosaurus catbaensis*) was discovered on Cat Ba Island in Cat Hai District, Hai Phong City, northeastern Vietnam, where it was assumed to be endemic (Ziegler et al. 2008). Preliminary population assessments of *G. catbaensis* revealed that its effective population size, defined as number of mature individuals, is much smaller than the suggested threshold values for minimal viable populations to maintain a stable population in the long term (Ngo et al. 2016; Nguyen et al. 2016, 2018; Reed et al. 2003; Traill et al. 2007).

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Fig. 1. New population. (A) Habitat of *Goniurosaurus catbaensis* on one offshore island in Ha Long Bay, Quang Ninh Province; (B) Microhabitat of *G. catbaensis* in Ha Long Bay; (C) Adult male; and (D) Adult female from Ha Long Bay. Photos: H.N. Ngo.

Even in undisturbed habitats, *G. catbaensis* occurs at low densities (Ngo et al. 2016; Nguyen et al. 2016, 2018).

The insular Cat Ba Tiger Gecko was found to be vulnerable to anthropogenic disturbances, and of high demand in pet markets as well as on Internet platforms (Ngo et al. 2016; Nguyen et al. 2018). In addition to poaching, habitat destruction for touristic purposes has dramatically increased the pressure on the wild *G. catbaensis* population. Consequently, the need for protection of the Cat Ba Tiger Gecko has received growing attention. Based on the first international population and trade investigations, this species has recently been listed in the IUCN Red List of Threatened Species as "Endangered" (Nguyen et al. 2016). The wild population is probably in peril due to its restricted distribution range, rising anthropogenic threats, and the lack of appropriate conservation measures. For the latter, detailed information on habitat requirements and the exact distribution of this species is essential, but such data are currently lacking. Ngo et al. (2016) recently suggested the potential occurrence of *G. catbaensis* on at least one more offshore island in Ha Long Bay.

To confirm this possibility, we investigated other small offshore islands in Ha Long Bay, Quang Ninh Province to locate populations of *G. catbaensis*, and predicted the overall availability of suitable habitats for the species in northeast Vietnam. In addition, the present study aimed to provide the first data on microhabitat selection of *G. catbaensis*. We assumed that differences in habitat use would occur between age classes and sexes, as they have

been observed in other lizards (Snyder et al. 2010; van Schingen et al. 2015).

Materials and Methods

Study areas: Study sites were selected based on our previous surveys on Cat Ba Island, Hai Phong City, and on photo documentation which gave evidence for the possible occurrence of *Goniurosaurus* on a small island in Ha Long Bay, Quang Ninh Province (Ngo et al. 2016). Cat Ba Island and adjacent islands comprise isolated limestone karst formations, which provide diverse habitats for a unique flora and fauna (Clements et al. 2006). Cat Ba Archipelago was recognized as the "Cat Ba Archipelago Biosphere Reserve" (CBBR) by the United Nations Educational, Scientific and Cultural Organization (UNESCO) in 2004 due to its significant ecosystem and biodiversity values (CBBR Authority 2013). Ha Long Bay was also twice recognized (in 1994 and 2000) by UNESCO as a World Heritage Site for the outstanding universal value of its landscape, geology, and geomorphology (The Management Department of Ha Long Bay 2014). Both areas are among the most popular tourist destinations in Vietnam, and face challenges from rapid tourism development.

Field surveys: Field surveys were conducted on Cat Ba Island between June and August 2014, May 2015, and during a short time in June 2016, which fell in the non-hibernation season of *Goniurosaurus* (Grismer et

al. 1999; Ngo et al. 2016). Furthermore, six offshore islands in Ha Long Bay, situated in close proximity to Cat Ba Archipelago, were surveyed in July 2016. Night excursions were conducted between 7:30 and 11:30 PM, when the lizards were found to be active (Ngo et al. 2016; Ziegler et al. 2008). To measure morphological characters, the animals were captured by hand and subsequently released at the same spot after checking and taking measurements.

Ecological analyses: Microhabitat data were recorded for each sighted *G. catbaensis*, including substrate types (classified as cliff, rock, branch, sand, or forest floor), perch height (vertical distance between captured animal and ground, in cm), percentage of vegetation or cave coverage, position (resting outside or inside cave), substrate surface condition (dry or wet), and activity (resting, feeding, or foraging). Air temperature and relative humidity were measured with a digital thermohygrometer (TFA Dostmann/Wertheim Kat. Nr. 30.5015), and substrate temperature and body surface temperature of animals were measured with an infrared thermometer (Measupro IRT20).

To identify intraspecific differences in microhabitat selection by *G. catbaensis*, individuals were classified into different age classes according to their snout-vent lengths (SVL): SVL < 85 mm = juvenile, SVL ≥ 85 mm and < 105 mm = sub-adult, and SVL ≥ 105 mm = adult (Ngo et al. 2016). Adults were differentiated between the sexes, as well as between gravid and non-gravid individuals. Sex of specimens was determined by the presence of the large swollen hemipenial bulges in males, while non-swollen in females.

A *t*-test, with $\alpha = 0.05$, was performed to determine differences in microhabitat parameters between age classes and sexes. Statistical analyses were performed with the program PAST, Version 2.17c (Hammer et al. 2001).

Morphological analyses: Morphometric measurements of captured individuals were taken with a digital caliper to the nearest 0.1 mm. In addition, two voucher specimens of the newly discovered populations in Ha Long Bay were collected, euthanized with ethylacetate, preserved in 70% ethanol, and deposited in the collections of the Vietnam National Museum of Nature (VNMN), Hanoi, Vietnam (VNMN 05423, VNMN 05424). Morphological characters were taken according to Ngo et al. (2016), Orlov et al. (2008), Yang and Chan (2015), and Ziegler et al. (2008).

Abbreviations of measurements are as follows: snout vent length (SVL) from tip of snout to vent; tail length (TaL) from vent to tip of tail; distance between axilla and groin (AG) from posterior edge of forelimb insertion to anterior edge of hind limb insertion; forelimb length (FoL) from axilla to tip of longest finger; hindlimb length (HiL) from groin to tip of longest finger; snout to eye distance (SE) from tip of snout to anterior-most point of eye; eye to ear distance (EE) from posterior margin of eye to posterior margin of ear; orbital diameter (OD) greatest diameter of orbit; ear diameter (ED) longest dimension of ear; internarial distance (IND) as distance between nares; anterior eye distance (AED) as distance between

anterior corners of eyelids; posterior eye distance (PED) as distance between posterior corners of eyelids; maximum head width (HW); maximum head height (HH); head length (HL) from tip of snout to posterior edge of occiput; pileus length (PL) from tip of snout to posterior scale of the head; and jaw length (JL).

Abbreviations of scalation are as follows: supralabials (SPL); infralabials (IFL); nasal scales surrounding nares (N); internasals (IN); gular scales bordering the internasals (PostIN); postmentals (PM); gular scales bordering the postmentals (GP); eyelid fringe scales or ciliaria (CIL); granular scales surrounding dorsal tubercles (GST); dorsal tubercle rows at midbody (DTR); paravertebral tubercles between limb insertions (TL); scales around midbody (MB); subdigital lamellae under the first finger (LF1) and the fourth finger (LF4); subdigital lamellae under the first toe (LT1) and the fourth toe (LT4); precloacal pores (PP); and postcloacal tubercles (PAT).

Molecular analyses: To confirm the taxonomic status of the newly collected *Goniurosaurus* from Ha Long Bay, Quang Ninh Province, a fragment of the mitochondrial 16S ribosomal gene was amplified, using the primer pair 16Sar and 16Sbr (Palumbi et al. 1991), for three samples (VNMN 05424 plus two small tissue samples from two released individuals, field numbers G8 and G12). Tissue samples were taken from the tail tips, which were disinfected before immediate release of the animals at the site of capture. DNA was extracted from tissue samples using the DNeasy blood and tissue kit, Qiagen (Redwood City, CA). The extracted DNA from the fresh tissue samples were amplified by PCR, with the PCR volume (21 µl) consisting of 10 µl of mastermix (Fermentas, Canada), 5 µl of water, 2 µl of each primer at 10 pmol/µl, and 2 µl of DNA. The PCR conditions were: 95 °C for five minutes to activate the taq; with 40 cycles at 95 °C for 30s, 50 °C for 45s, 72 °C for 60s; and the final extension at 72 °C for six minutes (Ngo et al. 2016).

PCR products were subjected to electrophoresis through a 1% agarose gel (UltraPure™, Invitrogen). Gels were stained for 10 minutes in 1x TBE buffer at 2 pg/ml of ethidium-bromide, and visualized under UV light. Successful amplifications were purified to eliminate PCR components using GeneJET™ PCR Purification Kit (Fermentas, Canada). Purified PCR products were sent to 1st Base (Selangor, Malaysia) for sequencing. Sequences were edited using the program Geneious v.7.1.8 (Kearse et al. 2012). After sequences were aligned using Clustal X v2 (Thompson et al. 1997), data were analyzed by Bayesian inference as implemented in MrBayes v3.2 (Ronquist et al. 2012). Settings for these analyses followed Le et al. (2006), except that the number of generations in the Bayesian analysis was increased to 1×10^7 . The optimal model for nucleotide evolution was set to GTR+I+G as selected by Modeltest v3.7 (Posada and Crandall 1998). The cutoff point for the burn-in function was set to 13 in the Bayesian analysis, as -lnL scores reached stationarity after 13,000 generations in both runs. Nodal support was evaluated using posterior probability in MrBayes v3.2. Uncorrected pairwise divergences were calculated in PAUP*4.0b10 (Swofford 2001).

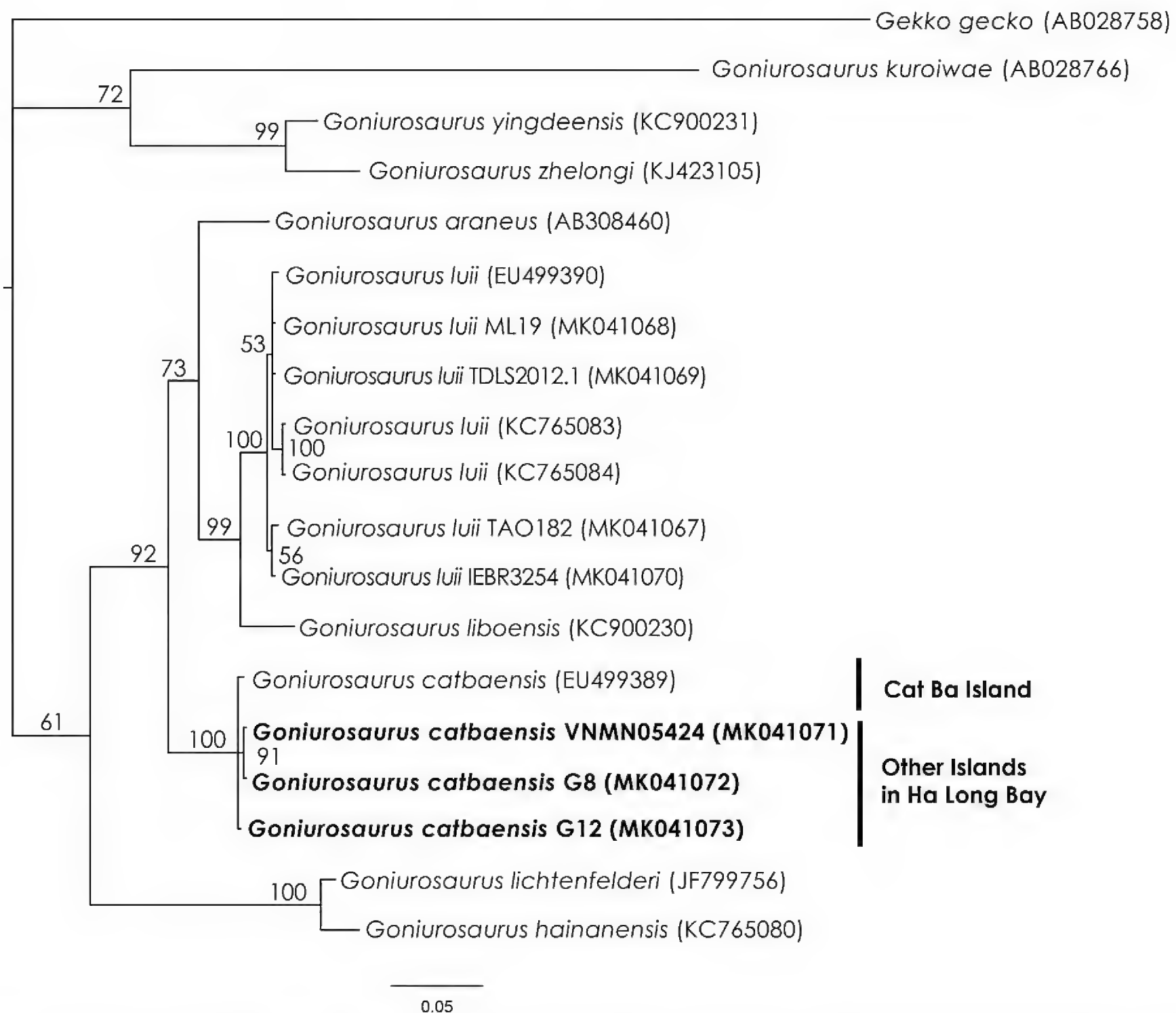


Fig. 2. Phylogram of *Goniurosaurus* based on the Bayesian analysis of a 16S ribosomal fragment. Numbers next to nodes are Bayesian posterior probabilities. Voucher numbers of new samples and GenBank accession numbers are placed after species names and in parentheses, respectively.

Species distribution models (SDMs): Based on occurrence records and a set of 19 environmental factors, the current overall availability of suitable habitats for *G. catbaensis* were predicted using the program Maxent Version 3.3.3.e (Beaumont et al. 2005; Phillips et al. 2006). Only the most distant occurrences of each site were included in the analyses to minimize effects of spatial autocorrelation and to ensure the independence of the records (Jennings and Veron 2011; Jennings et al. 2013). As a result, 11 records were filtered from a total of 60 localities of *G. catbaensis* on Cat Ba Island and Ha Long Bay. Nineteen bioclimatic variables that were obtained from the WorldClim global climate database (<http://www.worldclim.org>, accessed September 2016; Hijmans et al. 2005; Table 1) were used as environmental predictors.

Threat records: To get a first impression of trade in *Goniurosaurus* species in Vietnam, local pet markets were visited in Hai Phong and Ho Chi Minh cities, the two most important trade centers in the country, and different Internet platforms were investigated. Two local dealers from Ho Chi Minh City offering *Goniurosaurus* online were interviewed in September 2016, in order to trace the source of the traded *Goniurosaurus* species

in Vietnam. Additionally, five fishermen from the Ha Long Bay were interviewed to identify caves used by tourism companies for night parties, and determine the general attitude and use of the species in Ha Long Bay. Those sites located within the World Heritage Site were subsequently surveyed in July 2016 to evaluate potential threats from tourism activities. The names of interviewees are kept anonymous to ensure data privacy rights and Internet links are not disclosed to prevent misuse. Accurate locality data, cave names, and prices are also not presented to prevent targeted poaching for the wildlife trade.

Results

New records of *Goniurosaurus catbaensis*: During the present study, new *Goniurosaurus* occurrences were discovered on four small offshore islands, including two tourism caves in Ha Long Bay, Quang Ninh Province. The distances between these islands ranged from 1.4 km to 13 km, while the shortest distance between Cat Ba Island and one surveyed island in Ha Long Bay was 1.2 km. A total of 14 individuals (eight males, four females, one juvenile, and one unsexed individual which was only photographed) were recorded on these islands, which

Table 1. Bioclimatic variables from environmental data (Source: <http://www.worldclim.org>, accessed September 2016).

No.	Bioclimatic variables from the WorldClim dataset
1	BIO1 = “Annual Mean Temperature”
2	BIO2 = “Mean Diurnal Range” (Mean of monthly [max temp - min temp])
3	BIO3 = “Isothermality” (P2/P7) (*100)
4	BIO4 = “Temperature Seasonality” (standard deviation *100)
5	BIO5 = “Max Temperature of Warmest Month”
6	BIO6 = “Min Temperature of Coldest Month”
7	BIO7 = “Temperature Annual Range” (P5–P6)
8	BIO8 = “Mean Temperature of Wettest Quarter”
9	BIO9 = “Mean Temperature of Driest Quarter”
10	BIO10 = “Mean Temperature of Warmest Quarter”
11	BIO11 = “Mean Temperature of Coldest Quarter”
12	BIO12 = “Annual Precipitation (year)”
13	BIO13 = “Precipitation of Wettest Month”
14	BIO14 = “Precipitation of Driest Month”
15	BIO15 = “Precipitation Seasonality” (Coefficient of Variation)
16	BIO16 = “Precipitation of Wettest Quarter”
17	BIO17 = “Precipitation of Driest Quarter”
18	BIO18 = “Precipitation of Warmest Quarter”
19	BIO19 = “Precipitation of Coldest Quarter”

ranged between 0.34 and 2.94 km² in size.

Molecular analysis using Bayesian inference of the obtained matrix containing 613 aligned characters showed that all samples from Cat Ba Island (n = 1) and from the most distant other islands in Ha Long Bay (n = 3) clustered in a single clade with strong statistical support (posterior probability = 100%, Fig. 2). Genetic analyses revealed that sequences of the new records from Ha Long Bay, Quang Ninh Province, were identical to each other and virtually the same (99% to 100%) as that of the holotype of *G. catbaensis* from Cat Ba Island (GenBank accession number: EU499389). The maximum genetic divergence between the samples is approximately 0.3%, whereas the lowest divergence between two species of this genus, i.e., *G. hainanensis* and *G. lichtenfelderi*, is approximately 2.3% (Table 2). These results confirmed the newly recorded *Goniurosaurus* populations in Ha Long Bay are conspecific with *G. catbaensis* from Cat Ba Island (Fig. 2).

In addition, the morphological characters of the newly recorded *G. catbaensis* from Ha Long Bay accorded well with the population from Cat Ba Island, except that three of six individuals from a single site in Ha Long Bay showed a postrostral (internasal) scale. This character is consistently lacking in individuals recorded so far from Cat Ba Island (Ziegler et al. 2008) [Fig. 3A, 3B; Table 3].

Microhabitat selection: A total of 61 sightings took place (13 from smaller islands in the Ha Long Bay, and 48 from Cat Ba Island). *Goniurosaurus catbaensis* was active in the surroundings of large limestone caves covered in part by primary forest vegetation and in the vicinity of primary shrub vegetation on limestone. Mean air temperatures were 28.1 ± 1.7 °C (21.5–31.3 °C, n =

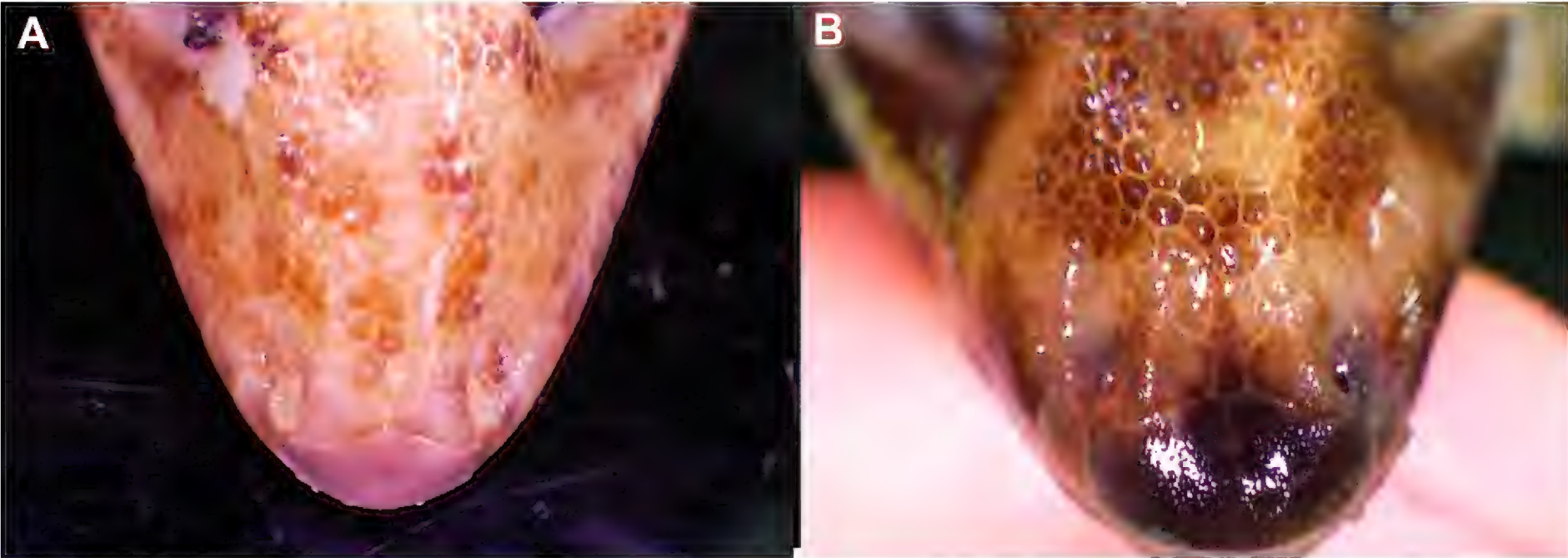


Fig. 3. Absence versus occasional presence of internasal scales of *Goniurosaurus catbaensis* from (A) Cat Ba Island and (B) Ha Long Bay. Photos H.N. Ngo.

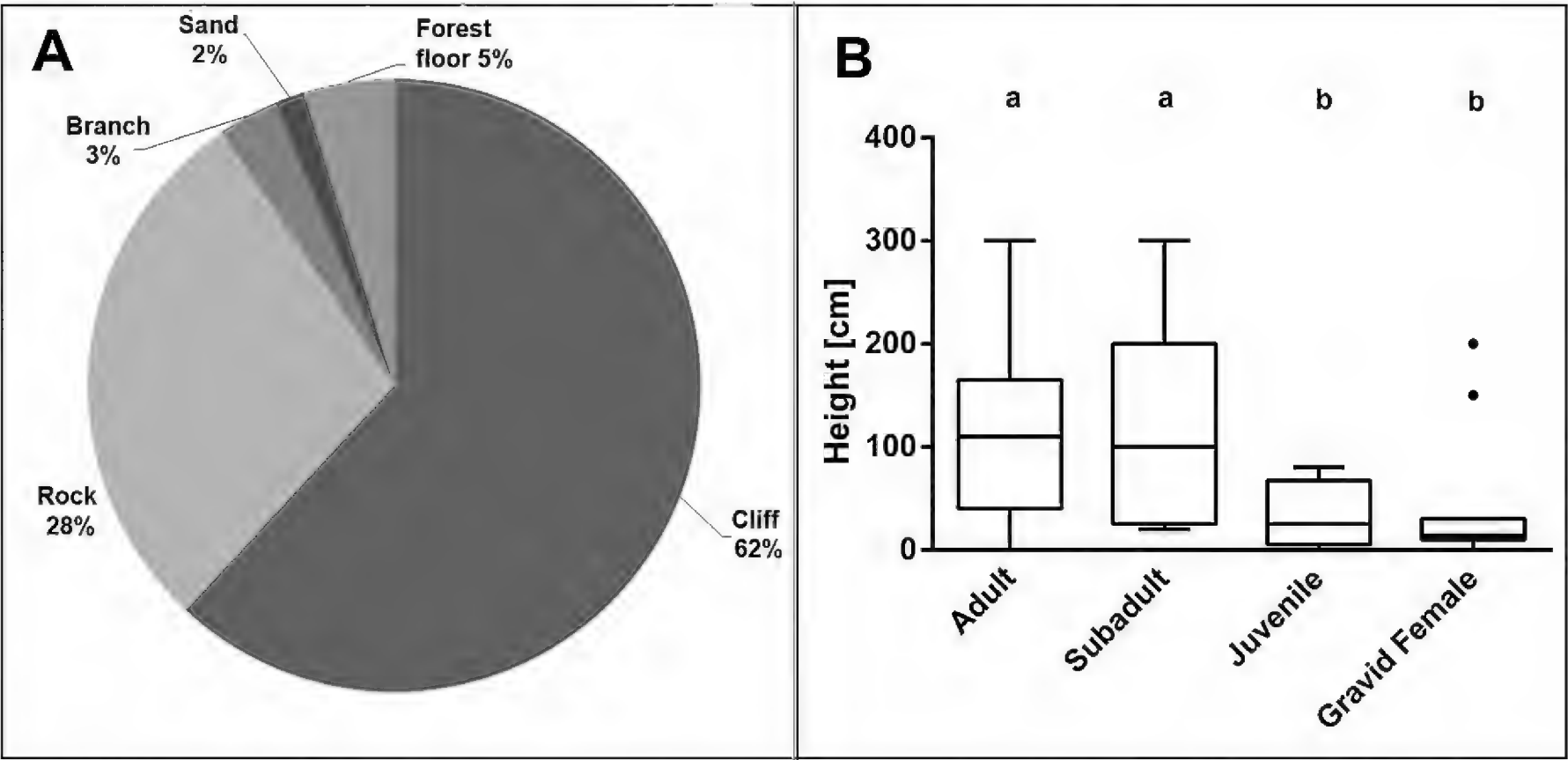


Fig. 4. (A) Substrate selection of *Goniurosaurus catbaensis*. (B) Box plots of perch heights of different age classes and sexes.

59) slightly higher than mean substrate temperatures of 26.02 ± 1.5 °C (22.2–28.2 °C, $n = 28$, Table 4). Recorded relative humidity at microsites ranged between 70–99% (mean $84.9 \pm 6.99\%$, $n = 52$).

A vast majority of lizards was found on limestone cliffs (62%), followed by rocks (28%), while only a few specimens were found on the forest floor (5%), branches (3%), or sand (2%) [Fig. 4A]. A significantly lower number of lizards was encountered inside compared to outside of limestone caves (26.9% vs. 73.1%, respectively). *Goniurosaurus catbaensis* selected spots with a mean canopy coverage of $95.2 \pm 9.6\%$ ($n = 63$, Table 4). Adult specimens (non-gravid) were found at average heights of 1.15 m ($n = 38$), while juveniles and gravid females resided at significantly lower heights of 0.28 m ($n = 4$) and 0.41 m ($n = 12$), respectively ($t = 2.82$, $df = 48$, $P < 0.05$; $t = 2.06$, $df = 40$, $P < 0.05$, Fig. 4B). A majority (about 77.4%, $n = 48$) of lizards was resting during the surveys, while only a few individuals ($n = 14$) were found actively foraging.

Suitable habitats for *G. catbaensis* were predicted to encompass a majority of small islands belonging to Cat Ba Island and Ha Long Bay, and include a wider area on the coastal mainland of Quang Ninh Province, where no surveys have been conducted so far (Fig. 5).

Trade: Trade in living tiger geckos has been frequently recorded by our team in local pet markets from Hai Phong and Ho Chi Minh cities, as well as on Facebook since 2015. Interviews with two local traders in Ho Chi Minh City revealed that they pay for local villagers living within the species’ distribution range to collect live tiger geckos during the non-hibernation season, confirming the wild (rather than captive-bred) source of traded animals. The dealers reportedly received individuals of three tiger gecko species, namely *G. huuliensis*, *G. luii*, and *G. catbaensis*, collected in April 2015. Among those, three individuals of *G. huuliensis* (one male and two females) were allegedly collected by a local hunter from Huu Lien Nature Reserve, Lang Son Province. Two local collectors from Cao Bang Province reportedly collected six individuals (three males and three females) of *G. luii* in northern Vietnam and another local hunter collected two couples of *G. catbaensis*. These 13 wild caught tiger geckos were transferred to pet markets in Ho Chi Minh City, southern Vietnam, in April 2015.

Human impacts on the habitat: Tourism activities in the region have dramatically increased in the past, and likely exerted enormous pressure on wild *G. catbaensis* populations. Events organized by tourism companies

Table 2. Uncorrected (“p”) distance matrix showing percentage pairwise genetic divergence (16S) between members of *Goniurosaurus*.

Species name	1	2	3	4	5	6	7	8	9
1. <i>G. araneus</i>	–								
2. <i>G. catbaensis</i>	6.4–6.7	–							
3. <i>G. hainanensis</i>	13.7	12.4–12.8	–						
4. <i>G. kuroiwae</i>	20.4	19.5–19.8	19.3	–					
5. <i>G. liboensis</i>	6.3	6.6–6.8	12.8	21.9	–				
6. <i>G. lichtenfelderi</i>	12.9	11.2–11.6	2.3	18.8	13.3	–			
7. <i>G. luii</i>	5.6–6.2	6.2–7.1	12.2–12.9	20.0–20.4	3.4–3.8	11.5–13.5	–		
8. <i>G. yingdeensis</i>	14.8	13.4–13.5	15.2	18.8	13.0	15.2	13.3–13.5	–	
9. <i>G. zhelongi</i>	15.3	14.2–14.4	16.9	21.4	13.4	16.2	14.8–15.4	4.8	–

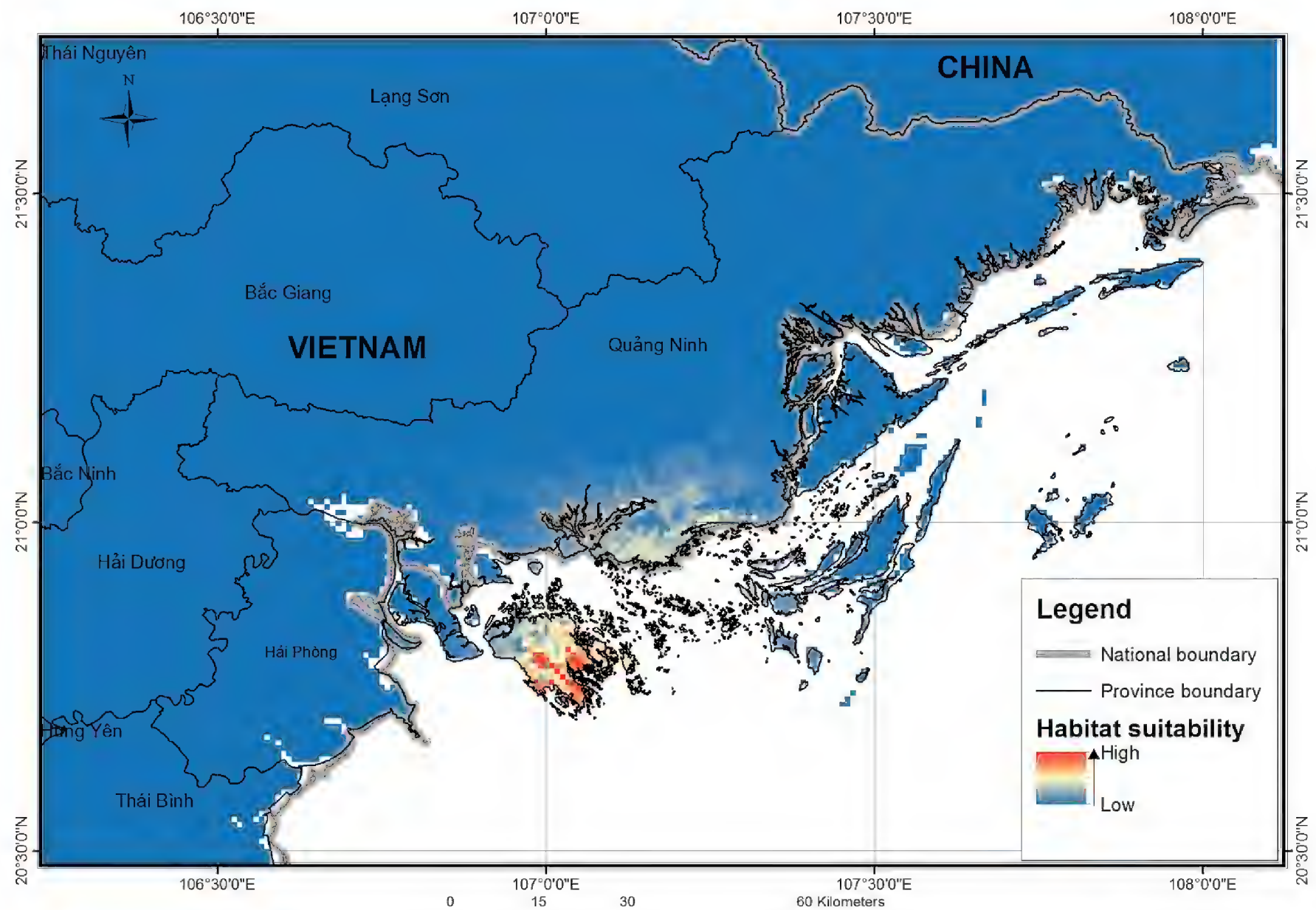


Fig. 5. Predicted habitat suitability for *Goniurosaurus catbaensis* in Vietnam.

regularly took place in at least two caves located within the UNESCO World Heritage Site. According to interviews with fishermen, daily excursions to the caves are scheduled to start at 7:30 PM and end around 11:00 PM. On these occasions, tourists dine in brightly lit caves before returning to their tour boats (Fig. 6B). As a consequence, wildlife is likely to be disturbed by the candle light, noisy sounds, and waste left by the tourists.

Discussion

New population records: Since its discovery in 2008, the Cat Ba Tiger Gecko was thought to be endemic to Cat Ba Island (Ziegler et al. 2008). These new records of *G. cf. catbaensis* on further offshore islands in Ha Long Bay confirmed for the first time the occurrence of the species outside its type locality. The newly recorded specimens showed an insignificant genetic divergence from the type series from Cat Ba Island and could be assigned to *G. catbaensis* (Table 2). Accordingly, the newly collected specimens from Ha Long Bay were also almost identical to the type series of *G. catbaensis* in morphology, except for the presence of a single internasal scale (which is absent in the type series from Cat Ba, see Ziegler et al. 2008) in a few individuals from a single site in Ha Long Bay. These findings indicated a slightly broader distribution range of the species than previously expected.

According to Li et al. (2010), the islands of Ha Long Bay and Cat Ba Archipelago were shaped by the erosion of limestone karst formations within the Gulf of Tonkin

at the northern east coast of Vietnam after the coastal shelf region became inundated by marine waters about 13,000 years ago. Repeated falls (> 50 m) of the sea level during glaciations periodically connected various islands and the mainland, which allowed exchanges between island and mainland populations, as well as colonization and re-colonization between island and mainland populations (Li et al. 2010; Liang et al. 2018). Thus, past recurrent gene flow is assumed to have occurred between (sub)populations, which helped to maintain a classical island-mainland metapopulation—in accordance with the high genetic similarity between *G. catbaensis* (sub)populations from different islands with identical habitats (Hanski 1991; Harrison and Taylor 1997; Levins 1969). Orlov et al. (2008) confirmed that *G. lichtenfelderi* was found from both continental mainland and islands. On the other hand, Liang et al. (2018) suggested that *G. lichtenfelderi* diverged from *G. hainanensis* of Hainan Island to Vietnam (including both mainland and island populations), which might have occurred during the glacial periods with past dispersal events. The speciation in the diversification process of *Goniurosaurus* was probably promoted by the adaption to different microhabitats. Populations of *G. lichtenfelderi* were found on granite beds of valley streams, while the closely related *G. hainaensis* is found on igneous rocks and *G. catbaensis* occurs in karst forests (Orlov et al. 2008; Liang et al. 2018; Ziegler et al. 2008; Nguyen et al. 2018).

To avoid the misuse of distribution data for targeted harvesting of the species (e.g., Lindenmayer and Scheele

Table 3. Morphological characters of *Goniurosaurus* from Ha Long Bay, Quang Ninh Province, compared with *G. catbaensis* from Cat Ba Island, Hai Phong Province. Measurements are given in mm. Note: (*) n = 6; (a) n = 2.

Specimens	Ha Long Bay (current study, n = 13)	Cat Ba Island (current study, n = 48)	Cat Ba Island (Ziegler et al. 2008) [n = 4]
SVL	74.54–122.7 (111.2 ± 11.9)	69.2–130.4 (108.9 ± 12.6)	84.7–111.5 (102.4 ± 14.5)
TaL	10.1–97.6 (69.9 ± 27)	28.9–104.02 (78 ± 17.7)	52.5–101.5 (68.1 ± 27.6)
AG	33.9–60.2 (52.9 ± 6.5)	43.07–58.43 (48.4 ± 5.4)*	-
HL	21.3–33.8 (30.2 ± 2.9)	17.8–34.2 (29.8 ± 3.5)	23.1–30.6 (27.7 ± 4.1)
HW	14.4–24.56 (22.1 ± 2.5)	13.9–28.2 (21.9 ± 2.5)	16.2–21.6 (19.5 ± 2.9)
HH	7.1–14.9 (12.8 ± 1.9)	8.2–16.9 (12.4 ± 1.9)	10.1–14.3 (12.2 ± 2.0)
FoL	32.2–53.8 (50.4 ± 5.6)	29.7–54 (47.8 ± 4.7)	-
HiL	42–67.47 (60.1 ± 6.2)	36.2–65 (57.9 ± 5.99)	-
SE	8.7–13.4 (11.9 ± 1.1)	10.45–13.4 (12.1 ± 1.0)*	9.8–12.6 (11.5 ± 1.6)
EE	9.4–13.2 (10.8 ± 1.5)*	9.78–12.13 (11.1 ± 0.88)*	8.5–12.3 (10.6 ± 2.1)
OD	5.6–8.3 (7.5 ± 0.7)	6.1–8.95 (7.6 ± 1.1)*	-
ED	2.8–5.3 (4.01 ± 0.8)	2.8–4.3 (3.5 ± 0.5)*	-
IND	3.39–4.33 (3.9 ± 0.34)*	2.9–4.2 (3.7 ± 0.5)*	-
IOD	6.78–8.62 (7.98 ± 0.67)*	6.9–8.47 (7.5 ± 0.6)*	-
ION	11.8–15.03 (13.9 ± 1.23)*	11.9–15.1 (13.1 ± 1.3)*	-
JL	12.3–10.8 (18.1 ± 2.1)	15.5–19.5 (17.2 ± 1.4)*	-
PL	27.6–32.5 (29.9 ± 1.7)*	26.6–32.8 (29.2 ± 2.5)*	-
SVL:HL	3.5–3.8 (3.68 ± 0.1)	3.3–4.3 (3.7 ± 0.2)	3.61–3.67 (3.7 ± 0.05)
SVL:AG	1.9–2.3 (2.1 ± 0.1)	2.04–2.45 (2.3 ± 0.14)*	-
HL:HW	1.28–1.48 (1.37 ± 0.05)	1.1–1.5 (1.36 ± 0.09)	1.43–2.11 (1.6 ± 0.4)
HL:HH	2.3–3.01 (2.35 ± 1.6)	1.79–3.3 (2.4 ± 0.4)	2.29–2.43 (2.33 ± 0.07)
SE:EE	0.9–1.2 (1.1 ± 0.1)*	1.07–1.1 (1.09 ± 1.14)*	1.02–1.15 (1.09 ± 0.07)
SPL	9–10 (9.4 ± 0.5)*	8–11 (10.08 ± 1.1)*	8–9 (8.7 ± 0.5)
IFL	8–9 (9.75 ± 0.45)*	8–10 (8.8 ± 0.7)*	6–8 (7.8 ± 0.6)
N	5–6 (5.25 ± 0.5) ^a	6–8 (7 ± 0.47)*	5–6 (5.1 ± 0.4)
IN	0–1 (0.23 ± 0.4)	0	0
PostIN	0–2 (0.4 ± 0.77)	0	0
PM	2–3 (2.5 ± 0.7) ^a	2–3 (2.83 ± 0.41)*	2–3 (2.8 ± 0.5)
GP	7 ^a	6–9 (7.8 ± 1.2)*	6–7 (7.22 ± 0.6)
CIL	45–49 (46.75 ± 1.7) ^a	41–56 (47.8 ± 4.4)*	52–55 (54.0 ± 1.1)
MB	104–109 (106.5 ± 3.5) ^a	102–109 (103.8 ± 3.8)*	112–127 (119.2 ± 7.6)
GST	9–12 (10.5 ± 1.3) ^a	9–14 (10.3 ± 1.6)*	8–11 (9.8 ± 1.6)
TL	35–37 (36 ± 1.4) ^a	27–34 (31.5 ± 3.0)*	33–34 (33.7 ± 0.6)
DTR	23 ^a	19–25 (22.3 ± 1.97)*	23–25 (24.0 ± 1.2)
LF1	9–12 (10.25 ± 1.3) ^a	9–11 (10 ± 0.7)*	11–12 (11.75 ± 0.5)
LF4	18–19 (18.75 ± 0.5) ^a	19–20 (19.3 ± 0.5)*	18–19 (18.1 ± 0.5)
LT1	9–10 (9.75 ± 0.5) ^a	9–10 (9.91 ± 0.3)*	11–12 (11.4 ± 0.6)
LT4	24 ^a	22–24 (23.4 ± 0.8)*	22–24 (23.4 ± 0.7)
PP	20–24 (22.5 ± 1.4)*	21*	5–21 (15.3 ± 2.5)
PAT	1–3 (2.25 ± 0.6)*	2–3 (2.5 ± 0.5)*	2–3 (2.8 ± 0.5)

2017; Stuart et al. 2006; Yang and Chan 2015), detailed locality information of the new records is being withheld. According to the SDMs *G. catbaensis* is predicted to occur on other, similar islands in the Gulf of Tonkin, but

is still endemic to Ha Long Bay and Cat Ba Archipelago. According to Orlov et al. (2008) the type locality of *G. lichtenfelderi* is an offshore island in Bai Tu Long Archipelago, which is contiguous with Ha Long Bay



Fig. 6. Potential threats to *Goniurosaurus*. **(A)** Flooding of Viet Hai Commune in August 2015. **(B)** Tourist event in a cave within the UNESCO World Heritage Site on Ha Long Bay. Photos H.N. Ngo.

in the Gulf of Tonkin. However, extensive field surveys have failed to record any individual of *G. catbaensis*, occurring in syntopy with *G. lichtenfelderi* (Gawor et al. 2016; Nguyen et al. 2011; Orlov et al. 2008). The habitat of *G. lichtenfelderi* in Bai Tu Long was described as valleys of forest streams on granite rocks within mixed forests of bamboo and broad-leaved trees (Gawor et al. 2016; Nguyen et al. 2009; Nguyen et al. 2011; Orlov et al. 2008; Ziegler et al. 2008), while *G. catbaensis* was found only in limestone karst ecosystems present in Ha Long and Cat Ba archipelagos. Accordingly, our SDMs predicted the potential distribution of *G. catbaensis* to encompass Ha Long Bay and Cat Ba Archipelago, but excluding Bai Tu Long Archipelago (Fig. 5). However, the present SDMs also predicted the mainland area including limestone formations around Ha Long City to be suitable for *G. catbaensis*. Thus, it will be important to search for further occurrences at these predicted sites in order to determine the exact distribution boundaries, and to assess genetic diversity of potentially new populations.

Microhabitat selection: Both sex- and age-related perch selection were found in *G. catbaensis*, namely differences in perch heights. Specifically, juveniles and gravid females occurred at significantly lower heights than subadults and adults. Similar habitat divergences between juveniles and adult individuals have been reported for Crocodile Lizards in Vietnam (van Schingen et al. 2015), and gekkonids in New Caledonia (Snyder et al. 2010).

This study also revealed that the body surface temperature of *G. catbaensis* showed a highly positive correlation with the air temperature ($r_s = 0.56$; $P < 0.05$,

$n = 23$) and substrate temperature ($r_s = 0.66$; $P < 0.001$, $n = 26$). Thus, as in other ectotherms, basic physiological functions of *G. catbaensis*, such as locomotion, growth, and reproduction are determined by the environmental temperature. Since tropical lizards are considered to have narrow temperature optima, and only few options for behavioral and physiological compensation, they are assumed to be especially vulnerable to extinction by climate warming (Deutsch et al. 2008; Doody and Moore 2010; Huey et al. 2009; Vié et al. 2009). In particular, body surface temperatures of *G. catbaensis* ranged from between 23.6 and 30.6 °C (mean = 27.2 ± 1.6 °C, $n = 26$) and were comparably higher than those of *G. kuroiwae* with average skin surface temperatures of 16.6 °C in the humid subtropical Oriental forest (Werner et al. 2005).

Potential threats and recommendations for conservation: Due to the restricted distribution range, low densities, and estimated global population being much lower than suggested threshold values for minimal viable populations, the Cat Ba Tiger Gecko is expected to be especially endangered to unsustainable for harvest (Ngo et al. 2016). Consequently, the species was recently assessed and ranked by the IUCN Red List of Threatened Species as "Endangered" (Nguyen et al. 2016). Other members of the genus *Goniurosaurus* from Vietnam have not been considered for inclusion on the IUCN Red List yet, as data on their population statuses are currently lacking. The findings reported here indicate that not only *G. catbaensis*, but also *G. huiliensis* and *G. luii*, are subject to intensive collection for local trade and provide concrete evidence for the wild source of the respective specimens. It is likely that the reported

Table 4. Environmental parameters characterizing the microhabitat selection of *Goniurosaurus catbaensis*.

Parameter	Number of sightings (n)	Min	Max	Mean $\pm$ SD
Canopy cover [%]	63	50	100	95.2 $\pm$ 9.6
Height [m]	54	0	3	0.97 $\pm$ 0.86
Elevation [m asl]	60	4	132	46.2 $\pm$ 32.9
Air Temperature [°C]	59	21.5	31.3	28.1 $\pm$ 1.7
Substrate Temperature [°C]	28	22.2	28.2	26.02 $\pm$ 1.5
Relative air Humidity [%]	52	70	99	84.9 $\pm$ 6.99

cases only reflect a small proportion of illegal harvesting activities. Since over-exploitation of local populations of range-restricted lizard species has been repeatedly found to rapidly cause extinction (e.g., Auliya et al. 2016; Stuart et al. 2006; Yang and Chang 2015), further research on the population status, distribution, ecology, and availability of suitable microhabitat sites is critically needed. The results of such studies may lead to the elevation or determination of the conservation status of other tiger gecko species and provide critical scientific data for future captive breeding programs. To reduce poaching and to control the trade in wild *Goniurosaurus*, we recommend continued monitoring of the scales and patterns of trade in combination with aforementioned population assessments. We also strongly advise against providing exact locality information for new *Goniurosaurus* populations in future publications, as this action might increase poaching activities at respective sites (Lindenmayer and Scheele 2017; Stuart et al. 2006; Yang and Chan 2015).

In addition to the illegal collection of animals, human impacts on habitats have dramatically increased by means of expanding tourism activities (see also Ngo et al. 2016). Tourism events in caves, causing disturbance by candle light, noisy sounds, and waste might result in the extirpation of *G. catbaensis* within these limestone caves. We suggest that tourism companies should hold such events only on their boats to reduce disturbances in the cave habitats of *G. catbaensis*, or at least restrict tourist access to only limited, selected islands.

Following Ngo et al. (2016), the sites in Viet Hai Village on Cat Ba Island had been recommended as a priority conservation zone for species conservation, since *G. catbaensis* was found to be most abundant at those sites. However, during the most recent survey in July 2016, no specimens of *G. catbaensis* were observed in Viet Hai Commune. We assume that an extensive flood in August 2015 might have killed a large amount of the local wildlife, including the Cat Ba Tiger Gecko, at this site. Viet Hai Commune was isolated for a week after torrential rains brought the water level up to the roofs of local houses. Since *G. catbaensis* was found to generally occur at low elevation ranges (4–132 m asl), and Viet Hai is situated only up to 36 m asl (see Fig. 6B), this species is particularly vulnerable to natural catastrophes such as storms, floods, and sea level rises, throughout its distribution range (see Dessler 2016; Saunders et al. 1991). Since local populations are extremely small, they are especially prone to extinction by catastrophic events. The devastating consequences of such natural disasters underline the importance of maintaining numerous independent subpopulations in order to compensate for such events.

In summary, the insular (sub)populations of *G. catbaensis* are threatened by harvest for the pet trade, human activities within its habitats, and natural catastrophes such as increasingly extreme floods and storms in northeastern Vietnam, probably triggered by climate change (The Governmental Committee on Flood and Storm Prevention 2016). Thus, we herewith emphasize the importance of setting aside priority conservation zones for this species, in order to establish

a connected and buffered system that allows (sub)populations to recover from catastrophes. We also recommend the establishment of an assurance population, i.e., an *ex situ* conservation breeding program for the species. Although such an effort has been started at the Me Linh Station for Biodiversity (see Ziegler et al. 2016) in Northern Vietnam, more resources need to be allocated to enhance the effort to conserve the species.

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Literature Cited

- Auliya M, Altherr S, Ariano-Sanchez D, Baard EH, Brown C, Cantu JC, Gentile G, Gildenhuis P, Henningheim E, Hintzmann J, et al. 2016. Trade in live reptiles and its impact on reptile diversity: the European pet market as a case study. *Biological Conservation* 204: 103–119.
- Beaumont LJ, Hughes L, Poulsen M. 2005. Predicting species distributions: use of climatic parameters in BIOCLIM and its impact on predictions of species' current and future distributions. *Ecological Modelling* 186: 250–269.
- Cat Ba Biosphere Reserve Authority. 2013. *Cat Ba Archipelago Biosphere Reserve*. Third edition. Hai Phong City, Vietnam. 28 p.
- Chen TB, Meng YJ, Jiang K, Li PP, Wen BH, Lu W, Lazell J, Hou M. 2014. New record of the Leopard Gecko *Goniurosaurus araneus* (Squamata: Eublepharidae) for China and habitat portioning between geographically and phylogenetically close leopard geckos. *IRCF Reptiles & Amphibians* 21(1): 16–27.
- Clements R, Sodhi NS, Schilthuizen M, Peter KLN-G. 2006. Limestone karsts of Southeast Asia: Imperiled arks of biodiversity. *BioScience* 56(9): 733–742.
- Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008. Impacts of climate warming on terrestrial ectotherms across

- latitude. *Proceedings of the National Academy of Sciences of the United States of America* 105: 6,668–6,672.
- Doody JS, Moore JA. 2010. Conceptual model for thermal limits on the distribution of reptiles. *Herpetological Conservation and Biology* 5: 283–289.
- Dessler A. 2016. *Introduction to Modern Climate Change*. Second edition. Cambridge University Press, New York, New York, USA. 267 p.
- Gawor A, Pham CT, Nguyen TQ, Nguyen TT, Schmitz A, Ziegler T. 2016. The herpetofauna of the Bai Tu Long National Park, northeastern Vietnam. *Salamandra* 52: 23–41.
- Grismer LL, Brian, EV, Viets E, Boyle LJ. 1999. Two new continental species of *Goniurosaurus* (Squamata: Eublepharidae) with a phylogeny and evolutionary classification of the genus. *Journal of Herpetology* 33(3): 382–393.
- Grismer LL, Ota H, Tanaka S. 1994. Phylogeny, classification, and biogeography of *Goniurosaurus kuroiwa* (Squamata: Eublepharidae) from the Ryukyu Archipelago, Japan, with description of a new subspecies. *Zoological Science* 11: 319–335.
- Hanski I. 1991. Single-species metapopulation dynamics. Pp. 17 In: *Metapopulation Dynamics: Empirical and Theoretical Investigations*. Editors, Gilpin ME, Hanski I. Academic Press, London, United Kingdom. 340 p.
- Hammer O, Harper DAT, Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. *Paleontologia Electronica* 4: 9.
- Harrison S, Taylor AD. 1997. Empirical evidence for metapopulation dynamics. In: *Metapopulation Biology: Ecology, Genetics and Evolution*. Editors, Hanski IA, Gilpin ME. Academic Press, San Diego, California, USA. 512 p.
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1,965–1,978.
- Honda M, Ota H. 2017. On the live coloration and partial mitochondrial DNA sequences in the topotypic population of *Goniurosaurus kuroiwa* *orientalis* (Squamata: Eublepharidae), with description of a new subspecies from Tokashikijima Island, Ryukyu Archipelago, Japan. *Asian Herpetological Research* 8: 96–107.
- Huey RB, Deutsch CA, Tewksbury JJ, Vitt LJ, Hertz PE, Alvarez Perez HJ, Garland T. 2009. Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B* 276: 1,939–1,948.
- Jennings AP, Mathai J, Brodie J, Giordano AJ, Veron G. 2013. Predicted distributions and conservation status of two threatened small carnivores: Banded Civet and Hose's Civet. *Mammalia* 77: 261–271.
- Jennings AP, Veron G. 2011. Predicted distributions and ecological niches of eight civets and mongoose species in Southeast Asia. *Journal of Mammalogy* 92: 316–327.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Coope A, Markowitz S, Duran C, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1,647–1,649.
- Le M, Raxworthy CJ, McCord WP, Mertz L. 2006. A molecular phylogeny of tortoises (Testudines: Testudinidae) based on mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* 40: 517–531.
- Levins R. 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America* 15: 237–240.
- Li Z, Zhang Y, Li Y, Zhao J. 2010. Palynological records of Holocene monsoon change from the Gulf of Tonkin (Beibuwan), northwestern South China Sea. *Quaternary Research* 74: 8–14.
- Liang B, Zhou RB, Liu YL, Chen B, Grismer LL, Wang N. 2018. Renewed classification within *Goniurosaurus* (Squamata: Eublepharidae) uncovers the dual roles of a continental island (Hainan) in species evolution. *Molecular Phylogenetics and Evolution* 127: 646–654.
- Lindenmayer D, Scheele B. 2017. Do not publish. *Science* 356(6340): 800–801.
- Ngo HN, Ziegler T, Nguyen TQ, Pham CT, Nguyen TT, Le MD, van Schingen M. 2016. First population assessment of two cryptic Tiger Geckos (*Goniurosaurus*) from northern Vietnam: Implications for conservation. *Amphibian & Reptile Conservation* 10(1) [General Section]: 34–45 (e120).
- Nguyen TQ. 2011. Systematics, ecology, and conservation of the lizard fauna in northeastern Vietnam, with special focus on *Pseudocalotes* (Agamidae), *Goniurosaurus* (Eublepharidae), *Sphenomorphus* and *Tropidophorus* (Scincidae) from this country. Ph.D. Dissertation, University of Bonn, Bonn, Germany. 229 p.
- Nguyen TQ, Ngo HN, van Schingen M, Ziegler T. 2016. *Goniurosaurus catbaensis*. *The IUCN Red List of Threatened Species*. Version 2016.2. Available: <http://www.iucnredlist.org> [Accessed: 5 March 2017].
- Nguyen TQ, Ngo HN, Ziegler T. 2018. The reptile fauna of the Cat Ba Archipelago: a unique biological resource with high level of local endemism and important conservation significance. Pp. 48–55 In: *Workshop Proceeding of Values and Solutions to Conserve Biodiversity in Ha Long Bay and Cat Ba Archipelago: 24 August 2017*, IUCN. International Union for Conservation of Nature, Gland, Switzerland.
- Nguyen VS, Ho TC, Nguyen TQ. 2009. *Herpetofauna of Vietnam*. Edition Chimaira, Frankfurt am Main, Germany. 768 p.
- Orlov NL, Ryabov SA, Nguyen TT, Nguyen TQ, Ho CT. 2008. A new species of *Goniurosaurus* (Sauria: Gekkota: Eublepharidae) from north Vietnam. *Russian Journal of Herpetology* 15(3): 229–244.
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991. *The Simple Fool's Guide to PCR*. University of Hawaii, Department of Zoology and Kewalo Marine Laboratory, Honolulu, Hawaii,

- USA. 94 p.
- Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190: 231–259.
- Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- Reed DH, O’Grady JJ, Brook BW, Ballou JD, Frankham R. 2003. Estimates of minimum viable population sizes for vertebrates and factors influencing those estimates. *Biological Conservation* 113: 23–34.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Saunders DA, Hobbs RJ, Margules CR. 1991. Biological consequences of ecosystem fragmentation: a review. *Conservation Biology* 5(1): 18–32.
- Seufer H, Kaverkin Y, Kirschner A. 2005. The genus *Goniurosaurus* Barbour, 1908 (Oriental Leopard Geckos). Pp. 133–175 In: *Die Lidgeckos: Pflege, Zucht, und Lebensweise*. Kirschner & Seufer Publishing, Karlsruhe, Germany. 238 p.
- Snyder J, Snyder L, Bauer AM. 2010. Ecological observations on the Gargoyle Gecko, *Rhacodactylus auriculatus* (Bavay, 1869), in southern New Caledonia. *Salamandra* 46: 37–47.
- Stuart BL, Rhodin AG, Grismer LL, Hansel T. 2006. Scientific description can imperil species. *Science* 312: 1,137.
- Swofford DL. 2001. *PAUP* Phylogenetic Analysis Using Parsimony (* and Other Methods), version 4*. Sinauer Associates, Sunderland, Massachusetts, USA.
- The Governmental Committee on Flood and Storm Prevention. 2016. Summary the performance of projects dealing with foods and storms in Vietnam.
- The Management Department of Ha Long Bay. 2014. Ha Long Bay Natural World Heritage. Second edition. Quang Ninh Province, Vietnam. 76 p.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: Xexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4,876–4,882.
- Traill LW, Bradshaw CJA, Brook BW. 2007. Minimum viable population size: a meta-analysis of 30 years of published estimates. *Biological Conservation* 139: 159–166.
- van Schingen M, Le MD, Ngo TH, Pham TC, Ha QQ, Nguyen TQ, Ziegler T. 2016. Is there more than one Crocodile Lizard? An integrative taxonomic approach reveals Vietnamese and Chinese *Shinisaurus crocodilurus* represent separate conservation and taxonomic units. *Der Zoologische Garten* 85: 240–260.
- van Schingen M, Pham CT, An HT, Nguyen TQ, Bernardes M, Bonkowski M, Ziegler T. 2015. First ecological assessment of the endangered Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 in Vietnam: microhabitat characterization and habitat selection. *Herpetological Conservation and Biology* 10: 948–958.
- Vié JC, Hilton-Taylor C, Stuart SN. 2009. *Wildlife in a Changing World – an Analysis of the 2008 IUCN Red List of Threatened Species*. International Union for the Conservation of Nature, Gland, Switzerland. 180 p.
- Werner YL, Takahashi H, Mautz WJ, Ota H. 2005. Behavior of the terrestrial nocturnal lizards *Goniurosaurus k. kuroiwae* and *Eublepharis macularius* (Reptilia: Eublepharidae) in a thigmothermal gradient. *Journal of Thermal Biology* 30: 247–254.
- Yang JH, Chan BP. 2015. Two new species of the genus *Goniurosaurus* (Squamata: Sauria: Eublepharidae) from southern China. *Zootaxa* 3980(1): 67–80.
- Zhou RB, Wang N, Chen B, Liang B. 2018. Morphological evidence uncovers a new species of *Goniurosaurus* (Squamata: Eublepharidae) from the Hainan Island, China. *Zootaxa* 4369(2): 281–291.
- Ziegler T, Nguyen TQ, Schmitz A, Stenke R, Rösler H. 2008. A new species of *Goniurosaurus* from Cat Ba Island, Hai Phong, northern Vietnam (Squamata: Eublepharidae). *Zootaxa* 1771: 16–30.
- Ziegler T, Rauhaus A, Mutschmann F, Dang PH, Pham CT, Nguyen TQ. 2016. Building up of keeping facilities and breeding projects for frogs, newts and lizards at the Me Linh Station for Biodiversity in northern Vietnam, including improvement of housing conditions for confiscated reptiles and primates. *Der Zoologische Garten* 85: 91–120.



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A new species of dwarf day gecko (Reptilia: Gekkonidae: *Cnemaspis*) from lower-elevations of Samanala Nature Reserve in Central massif, Sri Lanka

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Abstract.—A new day gecko species of genus *Cnemaspis* Strauch, 1887 is described from a midland forested area of Udamaliboda (north-western foothills of Samanala Nature Reserve) in Sri Lanka. This species is medium in size (30–35 mm SVL) and can be differentiated from all other Sri Lankan congeners by a suite of distinct morphometric, meristic, and color characters (dorsum with smooth and homogeneous granular scales; chin, gular, pectoral, and abdominal scales smooth; precloacal pores absent in males, 14–15 femoral pores separated by 9–11 unpored interfemoral scales in males; subcaudals smooth, subhexagonal, enlarged, subequal, forming a regular median row). It was recorded from tall trees with smooth bark in home gardens, and also on clay walls in very old tall houses in wet, cool, and shady forests, distributed across mid elevations (~450–650 m) with limited anthropogenic disturbance. They can climb to heights of 7 m on vertical surfaces of trees. The most noteworthy behavior of this species is that when “scared,” it runs only upward to the canopy of the tree or along the wall to hide within crevices. The major threats for this species in Udamaliboda and other locations in lower Samanala Nature Reserve are habitat loss due to expansion of commercial-scale agriculture and monoculture plantations, and illicit forest encroachments. Therefore, these foothill forests warrant special conservation, habitat protection, further in-depth research, and specific hands-on management practices.

Keywords. Arboreal, conservation, ecology, rainforest, redlist, taxonomy, Sripadha, threats

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Introduction

Sri Lanka’s wet zone, home to unique assemblages of floral and faunal communities with high endemism, is one of the smallest biodiversity hotspots in the world (Meegaskumbura et al. 2002; Gunawardene et al. 2007). Within the diverse reptile community of the island (~225 species), the diversity of geckos (family Gekkonidae) is remarkable; 54 species (in eight genera) have been described so far, accounting for 24% of the overall reptilian species richness (Somaweera and Somaweera 2009; de Silva and Ukuwela 2017). Of these, 44 species (~81%) are endemic and 45 species (~83%) are threatened (MoE-SL 2012, Karunarathna et al. 2019a,b). The genus *Cnemaspis* comprises 32 species in Sri Lanka and all of them are endemic (Batuwita et al. 2019; Karunarathna et al. 2019a,b; de Silva et al. 2019). Sri Lankan *Cnemaspis*

species represent two distinct evolutionary lineages, the *C. kandiana* and *C. podihuna* clades (Agarwal et al. 2017; Karunarathna et al. 2019b). The high species richness in Sri Lanka may be due to multiple possible colonization events from the Indian mainland with isolated *in situ* speciation (Agarwal et al. 2017).

During the past decade, the number of species recognized in the genus *Cnemaspis* globally has grown rapidly, reaching over 150 species (Grismer et al. 2014; Uetz et al. 2019; Karunarathna et al. 2019b), and *Cnemaspis* is now the second most speciose gecko genus in the Old World (Rösler et al. 2019). Though Sri Lanka is a small island, it harbors about 21% of the world’s *Cnemaspis* species, of which 90% have been described in the last two decades, including many described only recently (Karunarathna et al. 2019b). However, most of the *Cnemaspis* species from the dry and intermediate climatic zones

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of Sri Lanka are restricted to small isolated hillocks scattered in the lowlands (Karunarathna et al. 2019b). Future studies on the biogeography of *Cnemaspis* in Sri Lanka are expected to highlight the importance of these isolated habitats in generating and maintaining the diversity of these unique groups of geckos on the island. During a field excursion to Udamaliboda (northwest Samanala Nature Reserve), an unidentified *Cnemaspis* species was discovered that closely resembles *C. gemunu*, *C. godagedarai*, *C. phillipsi*, and *C. scalpensis*, and it is described here as a new species.

Materials and Methods

Specimen collection. Museum acronyms follow Sabaj Pérez (2015). The type material discussed in this paper is deposited in the National Museum of Sri Lanka (NMSL), Colombo. Specimens were hand-caught and photographed in life. Three specimens were euthanized using halothane, fixed in 10% formaldehyde for two days, washed in water, and transferred to 70% ethanol for long-term storage. Tail tips were collected (as tissue samples) before fixation in formaldehyde for future genetic analyses and stored in 95% ethanol under relatively cool conditions (20–25 °C). For comparison, we examined 402 *Cnemaspis* specimens (catalogued and uncatalogued) representing all recognized Sri Lankan species, including all type specimens housed at the NMSL and The Natural History Museum, London (BMNH). Specimens that formerly belonged to the Wildlife Heritage Trust (WHT) collection and bear WHT numbers are currently deposited in the NMSL, catalogued under their original numbers. Original specimens in this study were collected during a survey on lizards of Sri Lanka under permit numbers WL/3/2/1/14/12 and WL/3/2/42/18 (A and B), issued by the Department of Wildlife Conservation, and permit numbers FRC/5 and FRC/6, issued by the Forest Department of Sri Lanka. Additional information on morphology and natural history of Sri Lankan *Cnemaspis* species was extracted from the relevant literature (Bauer et al. 2007; Manamendra-Arachchi et al. 2007; Wickramasinghe and Munindradasa 2007; Vidanapathirana et al. 2014; Amarasinghe and Campbell 2016; Wickramasinghe et al. 2016; Batuwita and Udugampala 2017; Agarwal et al. 2017; Batuwita et al. 2019; Karunarathna et al. 2019b). Assignment of unidentified specimens to species was based on the presence of shared morphometric and meristic characters (Wickramasinghe et al. 2016; Batuwita and Udugampala 2017; Agarwal et al. 2017; Batuwita et al. 2019; Karunarathna et al. 2019b; de Silva et al. 2019).

Morphometric characters. Forty morphometric measurements were taken (to the nearest 0.1 mm) using a Mitutoyo digital Vernier calliper, and detailed observations of scales and other structures were made through Leica Wild M3Z and Leica EZ4 dissecting microscopes. The

following symmetrical morphometric characters were taken on the left side of the body: eye diameter (ED), horizontal diameter of eye ball; orbital diameter (OD), greatest diameter of orbit; eye to nostril length (EN), distance between anteriormost point of orbit and posterior border of nostril; snout length (ES), distance between anteriormost point of orbit and tip of snout; snout to nostril length (SN), distance between tip of snout and anteriormost point of nostril; nostril width (NW), maximum horizontal width of nostrils; eye to ear distance (EE), distance between posterior border of eye and anteriormost point of ear opening; snout to axilla distance (SA), distance between axilla and tip of snout; ear length (EL), maximum length of ear opening; interorbital width (IO), shortest distance between left and right supraciliary scale rows; inter-ear distance (IE) distance across head between the two ear openings; head length (HL), distance between posterior edge of mandible and tip of snout; head width (HW), maximum width of head between the ears and the orbits; head depth (HD), maximum height of head at level of eye; jaw length (JL), distance between tip of snout and corner of mouth; internarial distance (IN), smallest distance between inner margins of nostrils; snout to ear distance (SED), distance between tip of snout and anteriormost point of ear; upper-arm length (UAL), distance between axilla and angle of elbow; lower-arm length (LAL), distance from elbow to wrist with palm flexed; palm length (PAL), distance between wrist (carpus) and tip of longest finger excluding the claw; length of digits I–V of manus (DLM), distance between juncture of the basal phalanx with the adjacent digit and the tip of the digit, excluding the claw; snout-vent length (SVL), distance between tip of snout and anterior margin of vent; trunk length (TRL), distance between axilla and groin; trunk width (TW), maximum width of body; trunk depth (TD), maximum depth of body; femur length (FEL), distance between groin and knee; tibia length (TBL), distance from knee to ankle with heel flexed; heel length (HEL), distance between ankle (tarsus) and tip of longest toe (excluding the claw) with both foot and tibia flexed; length of pedal digits I–V (DLP), distance between juncture of basal phalanx with the adjacent digit and the digit tip, excluding the claw; tail length (TAL), distance between anterior margin of the vent and tail tip; tail base depth (TBD), maximum height of tail base; and tail base width (TBW), widest point of tail base.

Meristic characters. Twenty-nine discrete characters were observed and recorded using Leica Wild M3Z and Leica EZ4 dissecting microscopes on both left and right sides of the body (reported in the form L/R): numbers of supralabials (SUP) and infralabials (INF), between first labial scale and corner of the mouth; number of interorbital scales (INOS), between left and right supraciliary scale rows; number of postmentals (PM) bounded by chin scales, 1st infralabial on left and right and the mental; number of chin scales (CHS), scales touching medial

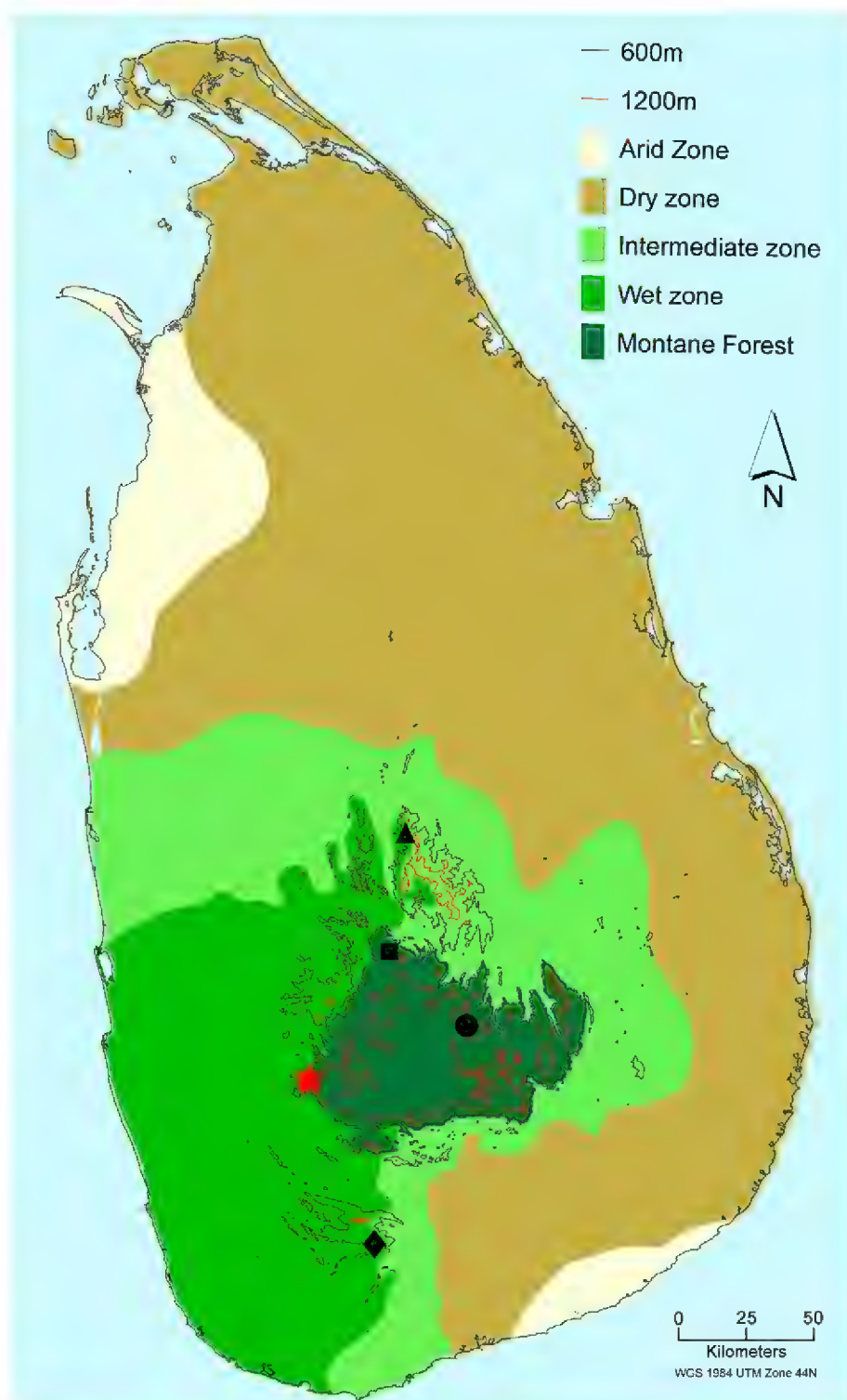


Fig. 1. Currently known distribution of *Cnemaspis anslemi* **sp. nov.** (Udamaliboda–star), and related species: *C. phillipsi* (Gammaduwa–triangle), *C. scalpensis* (Gannoruwa–square), *C. gemunu* (Haggala–circle), and *C. godagedarai* (Ensalwatte–diamond) in Sri Lanka.

edge of infralabials and mental between juncture of 1st and 2nd infralabials on left and right; number of supranasal (SUN), scales between nares; presence of postnasal (PON), scales posterior to naris; presence of internasal (INT), scale between supranasals; number of supraciliary scales (SUS), above eye; number of scales between eye and tympanum (BET), from posteriormost point of orbit to anteriormost point of tympanum; number of canthal scales (CAS), number of scales from posteriormost point of naris to anteriormost point of the orbit; total lamellae on manus I–V (SLM), counted from first proximal enlarged scansor greater than twice width of largest palm scale, to distalmost lamella at tip of digits; number of dorsal paravertebral granules (PG), between pelvic and pectoral limb insertion points along a straight line immediately left of vertebral column; number of midbody scales (MBS), from center of mid-dorsal row diagonally toward ventral scales; number of midventral scales (MVS), from first scale posterior to mental to last scale anterior to vent; number of belly scales (BLS), across venter between lowest rows of granular dorsal scales; total

lamellae on pes I–V (SLP), counted from first proximal enlarged scansor greater than twice the width of largest heel scale, to distalmost lamella at tip of digits; number of femoral pores (FP), present on femur; number of non-pored posterior femoral scales (PFS), counted from distal end of femoral pore row to knee; and interfemoral scales (IFS), number of non-pored scales between first femoral pores on both femurs. In addition, the texture (smooth or keeled) of ventral scales, the texture (homogeneous or heterogeneous) of dorsal scales, the number of spinous scales on flanks (FLSP), and characteristics such as appearance of caudal scales (except in specimens with regenerated tails) were also recorded. Coloration was determined from digital images of living specimens and also from direct observations in the field.

Natural history. The new species described here was collected on a field survey conducted in Udamaliboda, Samanala Nature Reserve of Sri Lanka (Fig. 1). Behavioral and other aspects of the natural history of the focal species were observed through opportunistic and non-systematic means. Such observations were made at a minimum distance of 2–4 m from the focal animals while taking precautions to avoid disturbances. To record elevation and georeference species locations, an eTrex® 20 GPS (Garmin) was used. Sex was determined by the presence (male) or absence (female) of femoral pores. The conservation status of the species was evaluated using the 2001 IUCN Red List Categories and Criteria version 3.1 (IUCN 2012).

Systematics

Cnemaspis anslemi **sp. nov.**

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Anslems' Day Gecko (English)

Anslemge divaseri hoona (Sinhala)

Anslemvin pahalpalli (Tamil)

Figs. 2–5; Tables 1–2

Holotype. NMSL.2019.14.01, adult male, 34.4 mm SVL (Fig. 2), collected from a tall, straight tree with good canopy cover in a home garden (bordering forest) in Udamaliboda, Kegalle District, Sabaragamuwa Province, Sri Lanka (6.859728°N, 80.448736°E, WGS1984; elevation 485 m, around 16.00 hrs) on 25 March 2019 by Suranjan Karunarathna and Kanishka Ukuwela.

Paratypes. NMSL.2019.14.02, adult female, 32.5 mm SVL collected from an old clay house wall (bordering forest) in Udamaliboda, Kegalle District, Sabaragamuwa Province, Sri Lanka (6.869611°N, 80.457069°E, WGS1984; elevation 634 m, around 10.00 hrs) on 26 March 2019 by Suranjan Karunarathna and Kanishka Ukuwela, and NMSL.2019.14.03, adult female, 30.0 mm SVL (Fig. 3) collected from a tall, straight tree with good canopy cover in a home garden (bordering the forest) in



Fig. 2. Close-ups of *Cnemaspis anslemi* **sp. nov.** male holotype (NMSL.2019.14.01): (A) dorsal, (B) lateral, (C) ventral aspects of head, (D) scales on lateral surface of trunk, (E) smooth ventral scales, (F) homogeneous dorsal scales, (G) cloacal characters with femoral pores, (H) subdigital lamellae on pes, (I) subdigital lamellae on manus, (J) lateral side of tail, (K) oval shaped subcaudals, and (L) dorsal scalation of tail. *Photos: Suranjan Karunaratna.*

Udamaliboda, Kegalle District, Sabaragamuwa Province, Sri Lanka (6.859728°N, 80.448736°E, WGS1984; elevation 485 m, around 14.00 hrs), on 27 March 2019 by Suranjan Karunaratna and Kanishka Ukuwela.

Diagnosis. *Cnemaspis anslemi* **sp. nov.** can be readily distinguished from its Sri Lankan congeners by a combination of the following morphological and meristic

characteristics, and also color pattern: maximum SVL 34.4 mm; dorsum with homogeneous, smooth granular scales; 2/2 supranasals, one internasal, and 1/1 postnasal present; three enlarged postmentals; postmentals bounded by five chin scales; chin and gular scales smooth, granular, juxtaposed; pectoral and abdominal scales smooth and subimbricate; 3–5 well developed tubercles on posterior flank; 118–122 paravertebral



Fig. 3. Dorsal and ventral aspects of the type series of *Cnemaspis anslemi* sp. nov. (A) Male holotype, NMSL.2019.14.01, (B) female paratype, NMSL.2019.14.02, and (C) female paratype, NMSL.2019.14.03 from Udamaliboda, Samanala Nature Reserve, Sri Lanka. Photos: Suranjan Karunarathna.

granules linearly arranged; 19–21 belly scales across venter; precloacal pores absent in males, 14–15 femoral pores on each side in males separated by 9–11 unpored interfemoral scales in males, and 2–3 unpored posterior femoral scales in males; 111–117 ventral scales; 87–91 midbody scales; subcaudals smooth, subhexagonal, enlarged, subequal, forming a regular median row; 8–9 supralabials; 8–9 infralabials; 16–17 total lamellae on digit IV of manus, and 20–21 total lamellae on digit IV of pes (Table 1). Dorsal body reticulated brown, black, and white; two large oval patches present on the neck; chin and gular with bright yellow, and femur dirty yellow.

Comparisons with other species. Based on the presence of enlarged hexagonal subcaudal scales *C. anslemi* sp. nov. can be assigned to the *C. podihuna* clade *sensu* Agarwal et al. (2017). However, the new species may be readily differentiated from congeners in this clade as follows: from *C. kandambyi* Batuwita and Udugampala, 2017, *C. molligodai* Wickramasinghe and Munindradasa, 2007, and *C. podihuna* Deraniyagala, 1944 by absence (*versus* presence) of precloacal pores; from *C. alwisi* Wickramasinghe and Munindradasa, 2007, *C. godagedarai* de Silva et al. 2019, *C. hitihami* Karunarathna et al. 2019, *C. kohukumburai* Karunarathna et al. 2019, *C. phillipsi* Manamendra-Arachchi et al. 2007, *C. punctata* Manamendra-Arachchi et al. 2007, *C. rajakarunai* Wickramasinghe et al. 2016, and *C. rammalensis* Vidanapathirana et al. 2014 by the presence of fewer ventral scales (111–117 *versus* 145–153, 133–137, 132–135, 131–134, 128–143, 129–137, 146–186, and 186–207, respectively); from *C. nilgala* Karunarathna et al. 2019 by the presence of more femoral pores (14–15 *versus* 7–9); from *C. gemunu* Bauer et al. 2007 by the presence of a greater number of belly scales (19–21 *versus* 13–16) and by presence of more paravertebral granules (118–122 *versus* 79–93); and from *C. scalpensis*

(Ferguson, 1877) by the presence of fewer tubercles on posterior flank (3–5 *versus* 9–11) and a greater number of paravertebral granules (118–122 *versus* 102–112).

Among species of the *C. kandiana* clade *sensu* Agarwal et al. (2017), *C. anslemi* sp. nov. differs by the absence (*versus* presence) of precloacal pores and the presence (*versus* absence) of clearly enlarged, hexagonal, or subhexagonal subcaudal scales from the following species: *C. amith* Manamendra-Arachchi et al. 2007, *C. butewai* Karunarathna et al. 2019, *C. gotaimbarai* Karunarathna et al. 2019, *C. ingerorum* Batuwita et al. 2019, *C. kallima* Manamendra-Arachchi et al. 2007, *C. kandiana* (Kelaart, 1852), *C. kivulegedarai* Karunarathna et al. 2019, *C. kumarasinghei* Wickramasinghe and Munindradasa, 2007, *C. latha* Manamendra-Arachchi et al. 2007, *C. menikay* Manamendra-Arachchi et al. 2007, *C. nandimithrai* Karunarathna et al. 2019, *C. pava* Manamendra-Arachchi et al. 2007, *C. pulchra* Manamendra-Arachchi et al. 2007, *C. retigalensis* Wickramasinghe and Munindradasa, 2007, *C. samanlensis* Wickramasinghe and Munindradasa, 2007, *C. silvula* Manamendra-Arachchi et al. 2007, *C. tropidogaster* (Boulenger, 1885) and *C. upendrai* Manamendra-Arachchi et al. 2007.

Description of Holotype. An adult male, 34.4 mm SVL. Body slender and relatively long (TRL 42.3% of SVL). Head relatively large (HL 30.3% of SVL, HL 71.6% of TRL), narrow (HW 17.2% of SVL, HW 56.7% of HL), depressed (HD 10.0% of SVL, HD 33.1% of HL) and distinct from neck. Snout relatively long (ES 80.7% of HW, ES 45.8% of HL), more than twice the eye diameter (ED 38.4% of ES), more than half the length of jaw (ES 67.8% of JL), snout slightly concave in lateral view; eye relatively small (ED 17.6% of HL), twice as large as ear (EL 34.4% of ED), pupil rounded; orbit length greater than eye to ear distance (OD 115.6% of EE) and greater

Table 1. Morphometric and meristic data of holotype and paratypes of *Cnemaspis anslemi* **sp. nov.** from Udamaliboda, Sri Lanka. Abbreviations: Holo–holotype, Para–paratype, M–male, F–female, L–left, R–right.

Measurement	NMSL 2019.14.01	NMSL 2019.14.02	NMSL 2019.14.03	Counts	NMSL 2019.14.01	NMSL 2019.14.02	NMSL 2019.14.03
	Holo (M)	Para (F)	Para (F)		Holo (M)	Para (F)	Para (F)
SVL	34.4	32.5	30.3	FLSP (L/R)	5/5	3/3	4/3
ED	1.8	1.8	1.8	SUP (L/R)	8/8	9/8	8/8
OD	3.3	3.3	3.1	INF (L/R)	9/8	8/8	8/8
EN	2.9	2.7	2.7	INOS	27	29	26
ES	4.8	4.7	4.6	PM	3	3	3
SN	1.3	1.1	1.1	CHS	5	5	5
NW	0.2	0.2	0.2	SUN (L/R)	2/2	2/2	2/2
EE	2.9	2.7	2.7	PON (L/R)	1/1	1/1	1/1
SA	16.9	15.1	15.2	INT	1	1	1
EL	0.6	0.6	0.6	SUS (L/R)	9/10	11/11	10/9
IO	3.4	3.3	3.3	BET (L/R)	18/18	18/17	19/18
IE	4.8	4.7	4.7	CAS (L/R)	9/10	8/8	9/8
HL	10.4	9.9	9.9	TLM (i) (L/R)	11/11	10/11	10/10
HW	5.9	5.8	5.7	TLM (ii) (L/R)	12/12	12/13	12/11
HD	3.5	3.1	3.0	TLM (iii) (L/R)	14/13	14/14	13/13
JL	7.0	6.9	6.9	TLM (iv) (L/R)	17/17	17/16	17/17
IN	1.7	1.8	1.7	TLM (v) (L/R)	13/13	13/13	13/12
SED	9.5	9.4	9.4	PG	122	118	121
UAL	5.1	4.9	4.9	MBS	87	91	90
LAL	5.2	5.1	5.1	MVS	117	112	111
PAL	4.6	5.7	5.9	BLS	21	19	19
DLM (i)	1.4	1.3	1.4	TLP (i) (L/R)	9/9	9/9	9/9
DLM (ii)	2.8	2.7	2.7	TLP (ii) (L/R)	13/12	12/12	12/13
DLM (iii)	3.1	2.9	2.9	TLP (iii) (L/R)	18/18	19/18	17/18
DLM (iv)	3.3	3.1	3.2	TLP (iv) (L/R)	21/21	21/20	21/21
DLM (v)	2.5	2.4	2.4	TLP (v) (L/R)	16/16	15/16	15/15
TRL	14.6	12.3	12.0	FP (L/R)	15/14	-	-
TW	6.3	6.1	6.2	PFS (L/R)	3/2	-	-
TD	3.8	3.9	3.9	IFS	10	-	-
FEL	7.1	6.9	6.9				
TBL	6.2	6.1	6.1				
HEL	6.2	6.3	6.2				
DLP (i)	2.2	2.1	2.2				
DLP (ii)	3.4	3.2	3.4				
DLP (iii)	3.8	3.8	3.7				
DLP (iv)	4.2	4.1	4.2				
DLP (v)	3.6	3.5	3.6				
TAL	39.4	36.5	34.7				
TBW	3.8	3.5	3.4				
TBD	3.1	2.9	2.9				

than the length of digit IV of the manus (OD 100.3% of DLM IV); supraocular ridges not prominent; ear opening very small (EL 6.0% of HL), deep, taller than wide, larger than nostrils; single row of scales separates orbit from supralabials; interorbital distance is narrow (IO 72.1% of ES), shorter than head length (IO 33.0% of HL); eye to

nostril distance slightly greater than eye to ear distance (EN 102.1% of EE).

Dorsal surface of the trunk with smooth, small homogeneous granules, 122 paravertebral granules; 117 smooth midventral scales; 87 midbody scales; 5/5 well developed tubercles on flanks; ventrolateral scales

slightly enlarged; granules on snout smooth and flattened, larger than those on interorbital and occipital regions; canthus rostralis not pronounced, 9/10 smooth oval scales from eye to nostril; scales of interorbital region oval and smooth; 2/2 weakly developed tubercles present on sides of neck and around ear; ear opening vertically oval, slanting from anterodorsal to posteroventral, 18/18 scales between anterior margin of the ear opening and posterior margin of eye. Supralabials 8/8 and infralabials 9/8, becoming smaller towards the gape. Rostral scale wider than long, partially divided (90%) by a median groove and in contact with first supralabial. Nostrils separated by 2/2 enlarged supranasals with one internasal and 1/1 postnasal; no enlarged scales behind supranasals. Nostrils oval, dorsolaterally oriented, not in contact with first supralabials.

Mental subrhomboidal, as wide as long, posteriorly in contact with three enlarged postmentals (smaller than mental, and larger than chin scales); postmentals in contact and bordered posteriorly by five smooth chin scales (larger than nostrils), contact with the 1st and 2nd infralabials; ventral scales smaller than chin scales, and larger than nostrils. Smooth, rounded, juxtaposed granule-like scales on chin and gular region; pectoral and abdominal scales smooth, subimbricate towards precloacal region, abdominal scales larger than dorsals; 21 belly scales across venter; smooth, subimbricate scales around vent and base of tail; 15/14 femoral pores; 10 unpored interfemoral scales; 3/2 small posterior femoral scales. Original tail of holotype longer than snout-vent length (TAL 114.5% of SVL); hemipenial bulge greatly swollen (TBW 3.8 mm), homogeneous scales on dorsal aspect of tail directed posteriorly, 1/1 spine-like tubercles present at base of tail, subcaudals very smooth; tail with 3–4 enlarged flattened obtuse scales forming whorls; absence of post-cloacal spur on each side; smooth subcaudals arranged into a median series of clearly enlarged, hexagonal or subhexagonal scales.

Forelimbs moderately short, slender (LAL 15.1% of SVL, UAL 14.8% of SVL) lower arm longer than upper arm; hind limbs moderately long, tibia shorter than femur (TBL 18.1% of SVL, FEL 20.5% of SVL). Dorsal, anterior, ventral, and posterior surfaces of upper arm with smooth scales, those on anterior surface twice as large as those on other faces of limb; dorsal, anterior, ventral, and posterior surfaces of lower arm with smooth scales, those on posterior surface twice as large as those of other parts; scales on dorsal surface of femur smooth and granular, less imbricate scales on anterior, posterior and ventral surfaces, scales on anterior surface are twice the size of those of other aspects. All surfaces of tibia with smooth scales; both anterior and posterior surfaces of limbs bearing smooth granules, scales of the ventral surface twice as large as those of other aspects. Dorsal and ventral scales on the manus and the pes smooth, granular; dorsal surfaces of digits with granular scales. Digits elongate and slender with inflected distal

phalanges, all bearing slightly recurved claws. Subdigital lamellae entire (except divided at first interphalangeal joint), unnotched; total lamellae on manus (left/right): digit I (11/11), digit II (12/12), digit III (14/13), digit IV (17/17), digit V (13/13); total lamellae on pes (left/right): digit I (9/9), digit II (13/12), digit III (18/18), digit IV (21/21), digit V (16/16); interdigital webbing absent; length order of digits of manus (left): I (1.4 mm), V (2.5 mm), II (2.8 mm), III (3.1 mm), IV (3.3 mm); length order of digits of pes (left): I (2.2 mm), II (3.4 mm), V (3.6 mm), III (3.8 mm), IV (4.2 mm).

Variation of the type series. The SVL of adult specimens in the type series ($n = 3$) and others ($n = 5$) ranges from 30.3 to 34.4 mm, TAL ranges from 34.7–39.4 mm, and TRL ranges from 12.0–14.6 mm; number of supralabials 8–9, and infralabials 8–9 (Table 1); spines on flank 3–5; interorbital scales 26–29; supraciliaries 9–11; canthal scales 8–10; scales from eye to tympanum 17–19; total lamellae on digits of manus: digit I (10–11), digit II (11–13), digit III (13–14), digit IV (16–17), digit V (12–13); total lamellae on digits of pes: digit II (12–13), digit III (17–19), digit IV (20–21), digit V (15–16); ventral scales 111–117, midbody scales 87–91; paravertebral granules 118–122; belly scales 19–21; unpored interfemoral scales 9–11 in males; femoral pores in males 14–15, and unpored posterior femoral scales in males 2–3.

Color of living specimens. The body color on the dorsal side is reddish brown; the dorsal head is randomly scattered with black and white dots; a yellowish oval patch on occiput, and a straight black middorsal dash over midpoint of neck (Fig. 4); faded yellow patches along vertebral midline; indistinct dark canthal stripe extends through eye and above ear, terminating anterior to forelimb insertion; the pupil of eye is circular and black with the surrounding being golden brown; a series of 4–5 mottled, irregular, dark brown transverse bands with gray margins on dorsum of body; dorsum of tail with 13–15 cinnamon brown blotches separating 12–14 faded dark brown bands; lateral view of labials and neck consists of thin black dots in bright yellow background like a zigzag mark; small dark spots (like eyes) present on back side of femur; chin and gular with bright yellow, vent and femur completely dirty yellow color.

Color of preserved specimens. Dorsum is light brown; dorsum of head is randomly scattered with brown and cream dots; an oval cream color patch on occiput, and a straight dark brown middorsal dash over midpoint of neck; a white post-orbital stripe present; labials with black and cream spots; venter is completely dirty white; tail with scattered markings on dorsal side.

Etymology. The specific epithet is an eponym Latinized (*anslemi*) in the masculine genitive singular, honoring the veteran Sri Lankan herpetologist Kongahage Anslem



Fig. 4. *Cnemaspis anslemi* sp. nov. male holotype (NMSL.2019.14.01) in life *in-situ*. (A) Dorsal view of the full body displaying the typical color pattern and a straight black middorsal dash over midpoint of neck, (B) Ventral aspect showing gular and femoral colorations, (C) lateral view showing labial coloration and zigzag pattern, (D) dorsal view of the full body of female paratype (NMSL.2019.14.02) in life *in-situ* from Udamaliboda, Samanala Nature Reserve, Sri Lanka. Photos: Kanishka Ukuwela and Suranjan Karunarathna.

Lawrence de Silva (the father of modern herpetology in Sri Lanka) for his valuable contributions to Sri Lankan herpetology and for inspiring the next generation of herpetologists, including the authors.

Natural history. The lower Samanala Nature Reserve area (along with Udamaliboda) comprises home gardens, and tropical evergreen rainforests (Gunatileke and Gunatileke 1990) mixed with tea and rubber plantations. The area comprises the Ratnapura and Kegalle districts and lies between 6.759172° and 6.889842°N and 80.436194° and 80.487717°E, at an elevation of 350–850 m. The mean annual rainfall varies between 3,500 and 4,500 mm, received mostly via the southwest monsoon (May–September). The mean annual temperature of the area is 26.4–27.9 °C. *Cnemaspis anslemi* sp. nov. is a quite rare species as six (± 0.1) geckos per survey-hour were found after covering a total area of 20 ha. This species was restricted to tall straight trees with smooth bark and thick canopy cover, and houses with tall clay walls with crevices. These geckos could climb up to 7 m on vertical surfaces of trees (Fig. 5). They were active during the day

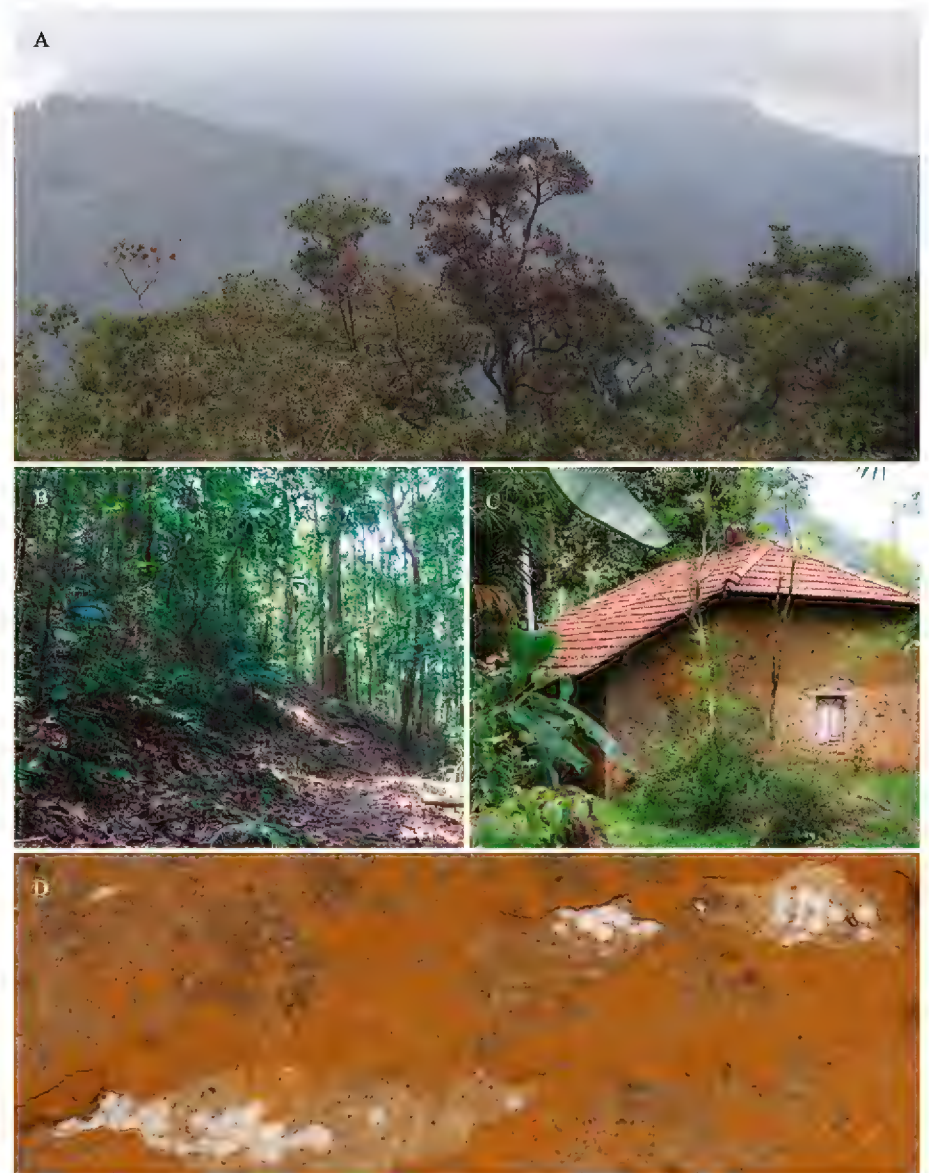


Fig. 5. General habitat of *Cnemaspis anslemi* sp. nov. at Udamaliboda, Samanala Nature Reserve, Kegalle District, Sri Lanka. (A) Complete view of the forest hill, (B) shady forest with thick leaf litter, (C) hundred years old house made using clay and bricks, also with wattle and daub, (D) communal egg laying site on a clay wall. Photos: Madhava Botejue and Suranjan Karunarathna.

time (08.00–17.00 h) and, when disturbed, sought refuge in tree tops with crevices. The new species was sympatric (at local habitat scale) with several other geckos (*Cnemaspis samanakensis*, *Cnemaspis* sp., *Cyrtodactylus triedrus*, *Cyrtodactylus* sp., *Gehyra mutilata*, *Hemidactylus depressus*, *H. pieresii*, *H. frenatus*, *H. parvimaculatus*, and *Hemiphyllodactylus typus*). The eggs were pure white in color and almost spherical in shape (~5 mm), with a slightly flattened side that attached to the clay-wall substrate. This species has also been recorded from the Lihinihela, Borangamuwa, and Warnagala areas in lower Samanala Nature Reserve.

Conservation status. Application of the IUCN Red List criteria indicates that *C. anslemi* sp. nov. is Critically Endangered (CR) due to having an area of occupancy (AOO) < 10 km² (six locations, 0.2 km² in total, assuming a 100 m radius around the georeferenced locations) and an extent of occurrence (EOO) < 100 km² (96.7 km²) in the lower elevations of Central Province [Applicable criteria are B2-b (iii)].

Remarks. *Cnemaspis anslemi* sp. nov. most closely resembles *C. gemunu*, *C. godagedarai*, *C. phillipsi*, and *C. scalpensis*. The type localities of these species are

Table 2. Key characters and identification features of 12 species belonging to the *podihuna* clade (*scalpensis* group), Sri Lanka. For these species, the dorsal scales are homogeneous, ventral scales are smooth, and subcaudal scales are clearly enlarged, hexagonal, or subhexagonal. Abbreviations: SVL—maximum snout to vent length in mm; SUP—supralabials; INF—infralabials; VEN—ventral scales; BEL—belly scales; FSP—spines on the flank; MBO—midbody scales; PVT—paravertebral scales; UPF—unpored interfemoral scales; FEP—femoral pores; LF4—lamellae on 4th finger; and LT4—lamellae on 4th toe.

Species	SVL	SUP	INF	VEN	BEL	FSP	MBO	PVT	UPF	FEP	LF4	LT4
<i>C. alwisi</i>	40.4	8–10	7–9	145–153	27–31	4–5	71–78	89–97	18–19	7–9	15–17	17–21
<i>C. anslemi</i>	34.4	8–9	8–9	111–117	19–21	3–4	87–91	118–122	9–11	14–15	16–17	20–21
<i>C. gemunu</i>	34.0	8–10	7–8	112–118	13–16	7–8	74–87	79–93	10–12	11–14	15–17	18–19
<i>C. godagedarai</i>	35.5	7–8	7–8	133–137	21–23	5–6	98–102	101–106	8–9	12–13	17–18	20–21
<i>C. hitihami</i>	41.7	8–9	7–9	132–135	21–22	4–5	96–99	143–149	24–26	5–10	18–19	21–22
<i>C. kohukumburai</i>	34.5	8–9	7–8	131–134	22–23	7–8	81–88	150–159	24–25	6–9	21–22	23–25
<i>C. nilgala</i>	32.9	7–8	6–7	122–129	17–19	3–4	71–78	179–187	14–15	7–9	17–18	17–18
<i>C. phillipsi</i>	36.6	8–9	8–9	128–143	18–25	4–6	76–91	86–93	11–14	15–16	16–19	17–19
<i>C. punctata</i>	39.9	7–10	7–9	129–137	20–29	11–13	71–78	83–91	25–27	5–7	17–18	17–23
<i>C. rajakarunai</i>	40.2	8–9	9–11	146–186	26–29	5–6	69–74	81–85	20–22	7–8	16–20	19–22
<i>C. rammalensis</i>	53.8	8–10	8–9	186–207	25–28	4–5	119–131	94–96	19–24	14–16	22–23	22–23
<i>C. scalpensis</i>	36.6	7–9	7–8	120–131	17–19	9–11	81–89	102–112	8–12	13–15	17–18	19–21

separated by ~38 km (Haggala in Nuwara Eliya), ~55 km (Ensalwatte in Deniyaya), ~83 km (Gammaduwa in Matale), and ~47 km (Gannoruwa in Kandy) airline distances, respectively, from Udamaliboda in Kegalle (Fig. 1). Further, the new species can be distinguished from *C. gemunu*, *C. godagedarai*, *C. phillipsi*, and *C. scalpensis* by morphometric and meristic characters (Table 2). We believe *Cnemaspis* cf. *gemunu* (AMB 7507, now in NMSL) collected from Borangamuwa in Ratnapura District (6.742778°N, 80.707778°E; elevation about 800 m) would most likely represent *C. anslemi* **sp. nov.** according to the currently known distribution pattern (see Agarwal et al. 2017). The records of *Cnemaspis scalpensis* from Udamaliboda forest and vicinity by Peabotuwage et al. (2012) also represent *Cnemaspis anslemi* **sp. nov.**

Discussion

The discovery and description of a novel species here adds yet another member to this speciose genus, increasing the known diversity of *Cnemaspis* in Sri Lanka to 33 species, all of which are endemic to the island. Several new descriptions during the last decade (e.g., Bauer et al. 2007; Manamendra-Arachchi et al. 2007; Wickramasinghe and Munindradasa 2007; Vidanapathirana et al. 2014; Wickramasinghe et al. 2016; Batuwita and Udu-gampala 2017; Batuwita et al. 2019; de Silva et al. 2019; Karunarathna et al. 2019a,b) have greatly advanced our knowledge on the diversity of these diminutive day geckos, increasing the total diversity from just four species. This trend most likely suggests that the diversity of Sri Lankan *Cnemaspis* is still underestimated, and further studies would most likely reveal more species from the varied natural and semi-natural habitats of Sri Lanka. Although Sri Lankan *Cnemaspis* are likely derived from the Indian radiation, the current diversity of this genus is

probably the result of multiple colonization events (poly-phyletic origin) as opposed to a single *in situ* radiation (monophyly); however, these phylogenetic and biogeographic affinities have yet to be confirmed (Agarwal et al. 2017; Bauer et al. 2007; Karunarathna et al. 2019b). We tentatively assign this species to the *podihuna* clade on the basis of clearly enlarged, hexagonal, or subhexagonal subcaudal scales (Fig. 6).

The preliminary studies reported here indicate that this novel species is frequently found in home garden habitats, as opposed to natural forest habitats, in the Udamaliboda region (Samanala Nature Reserve). During the survey from 2006 to 2019, only five specimens were found in the natural forest habitats of the Samanala Nature Reserve. The home gardens in which the new species was observed are heavily shaded and humid. The low encounter rates in natural forests could also be due to the low visibility conditions caused by the dense canopy, and this species may possibly occupy higher perches on the tree trunks, thus avoiding detection. However, further studies are necessary to ascertain this fact. Like most of the Sri Lankan *Cnemaspis* known so far (Bauer et al. 2007; Agarwal et al. 2017), the new species has a very small range, most likely due to the narrow ecological niche of this species (Slatyer et al. 2013). Its small size may reduce both dispersal ability as well as ecological tolerance levels. The Udamaliboda trail of Samanala Nature Reserve is inhabited by 11 species of geckos, including two undescribed species, two Critically Endangered, two Endangered, and two Vulnerable species (Peabotuwage et al. 2012). Overall, 65 reptile species have already been recorded from the type locality, indicating that the Udamaliboda is a local hotspot of reptile diversity.

The type locality of this species and the surrounding areas are currently subjected to encroachment via tea cultivation and mini-hydropower projects. Such activities will certainly reduce the crucial natural and semi-natural

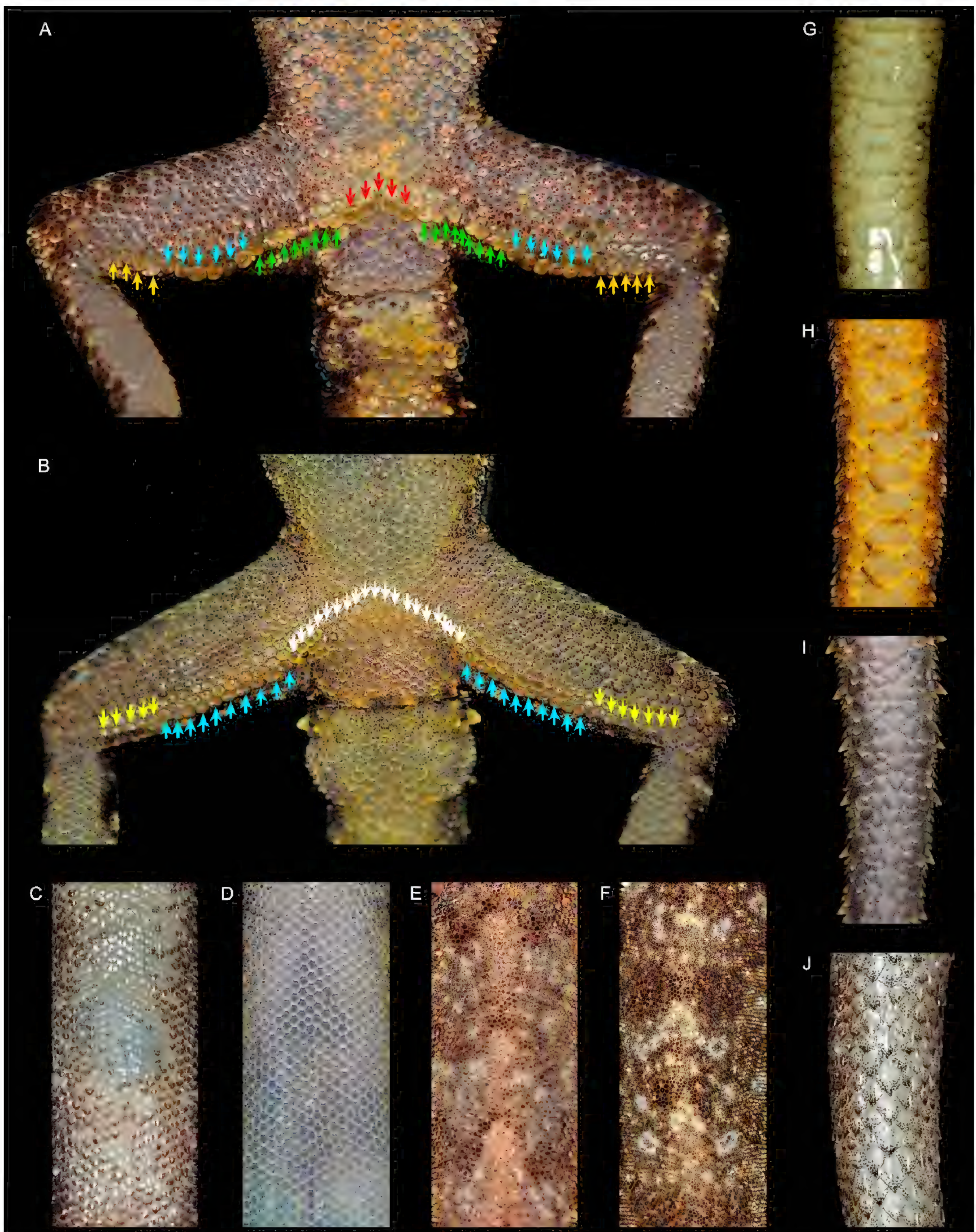


Fig. 6. Morphological characters that differentiate the species of *podihuna* and *kandiana* clades. (A) Scales and pores around vent in *podihuna* clade, (B) scales and pores around vent in *kandiana* clade, (C) keeled and imbricate belly scales, (D) smooth and imbricate belly scales, (E) heterogeneous keeled dorsal granules, (F) homogeneous smooth dorsal granules, (G) smooth sub-hexagonal subcaudals in *podihuna* clade, (H) smooth, solid hexagonal subcaudals in *podihuna* clade, (I) smooth, small, and irregular subcaudals in *kandiana* clade, (J) keeled, small, and irregular subcaudals in *kandiana* clade (yellow arrows: unpored posterior femoral scales in males; blue arrows: femoral pores in males; green arrows: unpored anterior femoral scales in males; red arrows: precloacal pores in males; white arrows: unpored interfemoral scales in males).

habitats of this range-restricted species. Thus, authorities should carefully consider new proposals for mini-hydro-power plants near or within natural habitats like these, in order to ensure that loss of the habitats occupied by such

rare or even undescribed species is minimized.

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Literature cited

- Agarwal I, Biswas S, Bauer AM, Greenbaum E, Jackman TR, de Silva A, Batuwita S. 2017. Cryptic species, taxonomic inflation, or a bit of both? New species phenomenon in Sri Lanka as suggested by a phylogeny of dwarf geckos (Reptilia, Squamata, Gekkonidae, *Cnemaspis*). *Systematics and Biodiversity* 15: 1–13.
- Amarasinghe AAT, Campbell PD. 2016. On the resolution of a long-standing issue surrounding the holotype of *Cnemaspis podihuna* Deraniyagala, 1944 (Reptilia: Gekkonidae). *Zootaxa* 4137: 296–300.
- Batuwita S, Udugampala S. 2017. Description of a new species of *Cnemaspis* (Squamata: Gekkonidae) from Knuckles Range of Sri Lanka. *Zootaxa* 4254: 82–90.
- Batuwita S, Agarwal I, Bauer AM. 2019. Description of a new diminutive, rupicolous species of day-gecko (Squamata: Gekkonidae: *Cnemaspis*) from southern Sri Lanka. *Zootaxa* 4565: 223–234.
- Bauer AM, de Silva A, Greenbaum E, Jackman T. 2007. A new species of day gecko from high elevation in Sri Lanka, with a preliminary phylogeny of Sri Lankan *Cnemaspis* (Reptilia: Squamata: Gekkonidae). *Mitteilungen aus dem Museum für Naturkunde, Berlin, Zoologische Reihe* 83: 22–32.
- Boulenger GA. 1885. *Catalogue of the Lizards in the British Museum (Natural History)*. British Museum of Natural History, London, United Kingdom. 436 p.
- de Silva A, Bauer AM, Botejue M, Karunarathna S. 2019. A new species of endemic day gecko (Reptilia: Gekkonidae: *Cnemaspis*) from a wet zone forest in second peneplain of Southern Sri Lanka. *Amphibian & Reptile Conservation* 13(1) [General Section]: 198–208 (e177).
- de Silva A, Ukuwela K. 2017. *A Naturalist's Guide to the Reptiles of Sri Lanka*. John Beaufoy Publishing, Oxford, United Kingdom. 144 p.
- Deraniyagala PEP. 1944. A new *Cnemaspis* gecko from Ceylon. *Journal of Royal Asiatic Society Sri Lanka* 97: 226–227.
- Ferguson WM. 1877. *Reptile Fauna of Ceylon. Letter on a Collection Sent to the Colombo Museum*. William Henry Herbert, Government Printer, Colombo, Ceylon (Sri Lanka). 42 p.
- Grismer LL, Wood PL Jr, Anuar S, Riyanto A, Ahmad N, Muin MA, Sumontha M, Grismer JL, Onn CK, Quah ESH, et al. 2014. Systematics and natural history of Southeast Asian rock geckos (genus *Cnemaspis* Strauch, 1887) with descriptions of eight new species from Malaysia, Thailand, and Indonesia. *Zootaxa* 3880: 1–147.
- Gunatilleke IAUN, Gunatilleke CVS. 1990. Distribution of floristic richness and its conservation in Sri Lanka. *Conservation Biology* 4: 21–31.
- Gunawardene NR, Daniels DA, Gunatilleke I, Gunatilleke C, Karunakaran P, Nayak GK, Prasad S, Puyravaud P, Ramesh B, Subramanian K. 2007. A brief overview of the Western Ghats–Sri Lanka biodiversity hotspot. *Current Science* 93: 1,567–1,572.
- IUCN. 2012. *IUCN Red List Categories and Criteria*. Version 3.1. 2nd edition. IUCN, Gland, Switzerland and Cambridge, United Kingdom. 32 p.
- Karunarathna S, Bauer A, de Silva A, Surasinghe T, Somaratna T, Madawala M, Gabadage D, Botejue M, Henkanaththegedara S, Ukuwela K. 2019a. Description of a new species of the genus *Cnemaspis* Strauch, 1887 (Reptilia: Squamata: Gekkonidae) from the Nilgala Savannah forest, Uva Province of Sri Lanka. *Zootaxa* 4545: 389–407.
- Karunarathna S, Poyarkov NA, de Silva A, Madawala M, Botejue M, Gorin VA, Surasinghe T, Gabadage D, Ukuwela K, Bauer AM. 2019b. Integrative taxonomy reveals six new species of day geckos of the genus *Cnemaspis* Strauch, 1887 (Reptilia: Squamata: Gekkonidae) from geographically isolated hill forests in Sri Lanka. *Vertebrate Zoology* 64: 247–298.
- Kelaart EF. 1852. *Prodromus Faunae Zeylanicae, being Contributions to the Zoology of Ceylon. Volume - 1*. Published by the author, Colombo, Sri Lanka. xxxiii + 197 + 54 + (3) p.
- Manamendra-Arachchi K, Batuwita S, Pethiyagoda R. 2007. A taxonomic revision of the Sri Lankan day geckos (Reptilia: Gekkonidae: *Cnemaspis*), with description of new species from Sri Lanka and Southern India. *Zeylanica* 7: 9–122.
- Meegaskumbura M, Bossuyt F, Pethiyagoda R, Manamendra-Arachchi K, Bahir M, Milinkovitch MC, Schneider CJ. 2002. Sri Lanka: an amphibian hotspot. *Science* 298: 379.
- MoE-SL. 2012. *The National Red List 2012 of Sri Lanka: Conservation Status of the Fauna and Flora*. Biodi-

- versity Secretariat, Ministry of Environment, Colombo, Sri Lanka. 476 p.
- Peabotuwage I, Bandara N, Samarasinghe D, Perera N, Madawala M, Amarasinghe C, Kandambi D, Karunarathna S. 2012. Range extension for *Duttaphrynus kotagamai* (Amphibia: Bufonidae) and a preliminary checklist of herpetofauna from the Uda Mälloboda Trail in Samanala Nature Reserve, Sri Lanka. *Amphibian & Reptile Conservation* 5(2): 52–64 (e38).
- Rösler H, Grismer LL, Ineich I, Kaiser H. 2019. The purportedly Indo-Australian gecko species *Cnemaspis timoriensis* (Duméril and Bibron, 1836) is actually the first named species of the neotropical genus *Gonatodes* Fitzinger, 1843 (Squamata: Gekkonidae). *Zootaxa* 4576: 483–509.
- Sabaj Pérez MH. 2015. Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an online reference, Version 5.0. American Society of Ichthyologists and Herpetologists, Lawrence, Kansas, USA. Available: <https://asih.org/standard-symbolic-codes> [Accessed: 22 June 2019].
- Slatyer RA, Hirst M, Sexton JP. 2013. Niche breadth predicts geographical range size: a general ecological pattern. *Ecology Letters* 16: 1,104–1,114.
- Somaweera R, Somaweera N. 2009. *Lizards of Sri Lanka: a Colour Guide with Field Keys*. Chimaira, Frankfurt am Main, Germany. 303 p.
- Uetz P, Freed P, Hošek J. 2019. The Reptile Database. Available: <http://reptile-database.reptarium.cz/> [Accessed: 20 July 2019].
- Vidanapathirana DR, Rajeev MDG, Wickramasinghe N, Fernando SS, Wickramasinghe LJM. 2014. *Cnemaspis rammalensis* sp. nov., Sri Lanka's largest day-gecko (Sauria: Gekkonidae: *Cnemaspis*) from Rammalakanda Man and Biosphere Reserve in southern Sri Lanka. *Zootaxa* 3755: 273–286.
- Wickramasinghe LJM, Munindradasa DAI. 2007. Review of the genus *Cnemaspis* Strauch, 1887 (Sauria: Gekkonidae) in Sri Lanka with the description of five new species. *Zootaxa* 1490: 1–63.
- Wickramasinghe LJM, Vidanapathirana DR, Rathnayake RMGP. 2016. *Cnemaspis rajakarunai* sp. nov., a rock dwelling day-gecko (Sauria: Gekkonidae: *Cnemaspis*) from Salgala, an unprotected lowland rainforest in Sri Lanka. *Zootaxa* 4168: 92–108.



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Appendix 1.

Comparative material:

Cnemaspis alwisi: NMSL 2004.09.01 (holotype), NMSL 2004.09.02 (paratype), NMSL 2004.09.03 (paratype), WHT 5918, WHT 6518, WHT 6519, WHT 7336, WHT 7337, WHT 7338, WHT 7343, WHT 7344, WHT 7345, WHT 7346.

C. amith: BMNH 63.3.19.1066A (holotype), BMNH 63.3.19.1066B (paratype), BMNH 63.3.19.1066C (paratype).

C. butewai: NMSL 2019.07.01 (holotype), NMSL 2019.07.02 (paratype), NMSL 2019.07.03 (paratype).

C. gemunu: AMB 7495 (holotype), AMB 7507 (paratype?), WHT 7221, WHT 7347, WHT 7348, NMSL 2006.11.01, NMSL 2006.11.02, NMSL 2006.11.03, NMSL 2006.11.04.

C. godagedarai: NMSL 2019.09.01 (holotype), NMSL 2019.16.01 (paratype), NMSL 2019.16.02 (paratype).

C. gotaimbarai: NMSL 2019.04.01 (holotype), NMSL 2019.04.02 (paratype), NMSL 2019.04.03 (paratype).

C. hitihami: NMSL 2019.06.01 (holotype), NMSL 2019.06.02 (paratype), NMSL 2019.06.03 (paratype).

C. ingerorum: WHT 7332 (holotype), WHT 7330 (paratype), WHT 7331 (paratype).

C. kallima: WHT 7245 (holotype), WHT 7222 (paratype), WHT 7227 (paratype), WHT 7228 (paratype), WHT 7229 (paratype), WHT 7230 (paratype), WHT 7239 (paratype), WHT 7249 (paratype), WHT 7251 (paratype), WHT 7252 (paratype), WHT 7253 (paratype), WHT 7254 (paratype), WHT 7255 (paratype).

C. kandambyi: WHT 9466 (holotype), WHT 9467 (paratype).

C. kandiana: BMNH 53.4.1.1 (lectotype), BMNH 80.2.2.119A (paralectotype), BMNH 80.2.2.119B (paralectotype), BMNH 80.2.2.119C (paralectotype), WHT 7212, WHT 7213, WHT 7267, WHT 7305, WHT 7307, WHT 7308, WHT 7310, WHT 7313, WHT 7319, WHT 7322.

C. kivulegedarai: NMSL 2019.08.01 (holotype), NMSL 2019.08.02 (paratype), NMSL 2019.08.03 (paratype).

C. kohukumburai: NMSL 2019.05.01 (holotype), NMSL 2019.05.02 (paratype), NMSL 2019.05.03 (paratype).

C. kumarasinghei: NMSL 2006.13.01 (holotype), NMSL 2006.13.02 (paratype).

C. latha: WHT 7214 (holotype).

C. menikay: WHT 7219 (holotype), WHT 7218 (paratype), WHT 7349 (paratype).

C. molligodai: NMSL 2006.14.01 (holotype), NMSL 2006.14.02 (paratype), NMSL 2006.14.03 (paratype), NMSL 2006.14.04 (paratype), NMSL 2006.14.05 (paratype).

C. nandimithrai: NMSL 2019.01.01 (holotype), NMSL 2019.01.02 (paratype), NMSL 2019.01.03 (paratype).

C. nilgala: NMSL 2018.07.01 (holotype), NMSL 2018.06.01 (paratype), NMSL 2018.06.02 (paratype), NMSL 2018.06.03 (paratype).

C. pava: WHT 7286 (holotype), WHT 7281 (paratype), WHT 7282 (paratype), WHT 7283 (paratype), WHT 7285 (paratype), WHT 7288 (paratype), WHT 7289 (paratype), WHT 7290 (paratype), WHT 7291 (paratype), WHT 7292 (paratype), WHT 7293 (paratype), WHT 7294 (paratype), WHT 7295 (paratype), WHT 7296 (paratype), WHT 7297 (paratype), WHT 7298 (paratype), WHT 7299 (paratype), WHT 7300 (paratype), WHT 7301 (paratype), WHT 7302 (paratype).

C. phillipsi: WHT 7248 (holotype), WHT 7236 (paratype), WHT 7237 (paratype), WHT 7238 (paratype).

C. podihuna: BMNH 1946.8.1.20 (holotype), NMSL 2006.10.02, NMSL 2006.10.03, NMSL 2006.10.04.

C. pulchra: WHT 7023 (holotype), WHT 1573a (paratype), WHT 7011 (paratype), WHT 7021 (paratype), WHT 7022 (paratype).

C. punctata: WHT 7256 (holotype), WHT 7223 (paratype), WHT 7226 (paratype), WHT 7243 (paratype), WHT 7244 (paratype).

C. rajakarunai: NMSL 2016.07.01 (holotype), DWC 2016.05.01 (paratype), DWC 2016.05.02 (paratype).

C. rammalensis: NMSL 2013.25.01 (holotype), DWC 2013.05.001.

C. retigalensis: NMSL 2006.12.01 (holotype), NMSL 2006.12.02 (paratype), NMSL 2006.12.03 (paratype), NMSL 2006.12.04 (paratype).

C. samanaleensis: NMSL 2006.15.01 (holotype), NMSL 2006.15.02 (paratype), NMSL 2006.15.03 (paratype), NMSL 2006.15.04 (paratype), NMSL 2006.15.05 (paratype).

C. scalpensis: NMSL 2004.01.01 (neotype), NMSL 2004.02.01, NMSL 2004.03.01, NMSL 2004.04.01, WHT 7265, WHT 7268, WHT 7269, WHT 7274, WHT 7275, WHT 7276, WHT 7320.

C. silvula: WHT 7208 (holotype), WHT 7206 (paratype), WHT 7207 (paratype), WHT 7209 (paratype), WHT 7210 (paratype), WHT 7216 (paratype), WHT 7217 (paratype), WHT 7018, WHT 7027, WHT 7202, WHT 7203, WHT 7220, WHT 7354, WHT 7333.

C. tropidogater: BMNH 71.12.14.49 (lectotype), NMSL 5152, NMSL 5151, NMSL 5159, NMSL 5157, NMSL 5970, NMSL 5974.

C. upendrai: WHT 7189 (holotype), WHT 7184 (paratype), WHT 7187 (paratype), WHT 7188 (paratype), WHT 7181 (paratype), WHT 7182 (paratype), WHT 7183 (paratype), WHT 7185 (paratype), WHT 7190 (paratype), WHT 7191 (paratype), WHT 7192 (paratype), WHT 7193 (paratype), WHT 7194 (paratype), WHT 7195 (paratype), WHT 7196 (paratype), WHT 7197 (paratype), WHT 7260 (paratype).



Book Review

How Snakes Work: Structure, Function and Behavior of the World's Snakes

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Keywords. Reptilia, Squamata, Ophidia, anatomy, locomotion, physiology, reproduction, thermoregulation

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“They are magnificent. They are splendid creations ingeniously constructed after eons of gradual change.” Kauffeld, *Snakes and Snake Hunting*, 1957

There are many books about snakes for every level of understanding, but rarely, if ever, has there been a book about snakes that matches Harvey Lillywhite's offering. As I went through the volume, I kept thinking how I wish I had such a book when I was in high school/college, or even as a kid. I wrote a term paper in high school on how snakes move and this book has an entire chapter devoted to it! I honestly got goosebumps and bursts of adrenaline as I read it, because it brought me back to my younger days of discovery about the biology of snakes. How fascinating they were/are! Lillywhite's volume details all the reasons why snake are magnificent organisms.

The volume is in a comfortable 8 1/2 by 11-inch format with 241 pages, glossary, index, and references at the end of each chapter. Rick Shine wrote the Foreword and Lillywhite explains in a short Preface his personal reasons and goals for the book. Lillywhite hoped that he “might bring a unique perspective to the subject matter, and to cover a range of topics...that will interest a wide group of readers.” I think he is completely successful in reaching those goals. Perhaps the reason he is so successful—and why this is such a terrific book—is because the author is an insider, a professional research scientist who has studied how snakes work for his entire career. We are all lucky that Lillywhite is sharing his expertise and passion with us in such a volume.

There are nine chapters: 1) Evolutionary History and Classification, 2) Feeding, Digestion, and Water Balance, 3) Locomotion: How Snakes Move, 4) Temperature

and Ectothermy, 5) Structure and Function of Skin, 6) Internal Transport: Circulation and Respiration, 7) Perceiving the Snake's World: Structure and Function of Sense Organs, 8) Sound Production, and 9) Courtship and Reproduction. There is obviously a lot of information here and it is as up to date as it could be. Each of the chapters is wonderfully adorned with terrific photos and illustrations that help the reader grasp and/or visualize the text descriptions.

Even in a book on how snakes work, a chapter on evolution and classification is practically obligatory because it puts the rest of the book's information into context. Maybe the most important nugget of knowledge given in this chapter is “The latter [snakes] are essentially highly derived lizards.” It is critical to point out that snakes are not related to lizards, but *are* lizards. That realization makes all the novel evolutionary changes relative to lizards that are described in this book even more remarkable. With that fact written in the book, it is somewhat unfortunate that an accompanying figure shows snakes and lizards as separate branches on a phylogeny of the tetrapods. The natural history comments in the “tip toe through the taxa” section of the chapter are a welcome addition.

An example of how having a volume written by a professional research scientist enhances the content is near the beginning of Chapter 2. Lillywhite wrote, “The following discussion is essentially speculative, but it provides a conceptual basis from which to launch considerations of feeding and digestion in snakes.” Because of his long history of work in the field, his speculations are valuable and make you feel like you just asked an expert a question and he is giving you

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his unique insight. I consider it a bonus. Beyond the speculation, this chapter is a trove of information on the diversity of feeding adaptations among snakes, such as the physiology required to handle digestion of various food types, and the myriad aspects of how snakes physiologically deal with water.

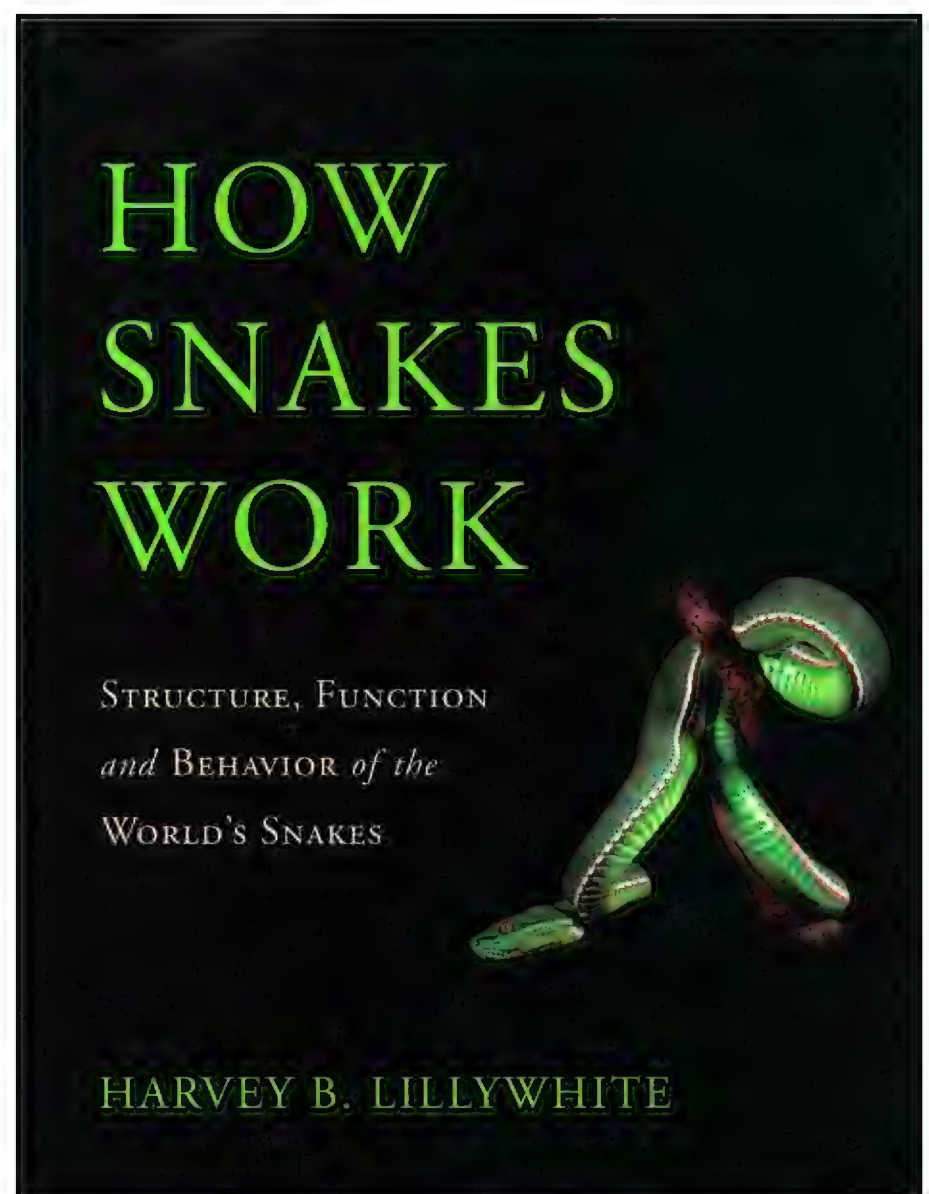
So, you think you know how snakes move? In my high school term paper that I previously mentioned, I described three modes: lateral, concertina, and rectilinear motion. I was woefully incomplete. The illustrations in Harvey's book, from anatomy to movement, are excellent and useful. The final section of this chapter, titled *Snakes as Robots*, is a treat.

Of course, we recognize snakes as ectotherms, but as Lillywhite reveals in Chapter 4 on "Temperature and Ectothermy," being an ectotherm is an extremely complicated deal for snakes. The chapter goes through these many complications and even provides examples of **endothermy** in snakes, which makes the label "ectotherm" require an even more broad interpretation. Lillywhite does a great job pointing out how much is not known about thermoregulation, especially with regard to trade-offs or cost-benefits when thermoregulation is coupled with any of the myriad of other physiological processes of the organism.

There is something magical about seeing snake skin that is either as glossy as glass, like mud snakes (*Farancia abacura*), or spectacularly iridescent, like rainbow boas (*Epicrates cenchria*). Well the chapter on skin covers it all and tells you everything you need (or wanted) to know about snake skin, from cultural references to histology and micrographs to mimicry and color/pattern variation. As usual for this book, the photos and illustrations are terrific!

The concluding paragraph of the chapter on Internal Transport opens with: "Few persons probably give much thought to how the blood circulates within a snake and how the lung functions to assist in the delivery of oxygen." I have to agree with Lillywhite here, but I also have to add that after reading the chapter one can't help but learn things, even if you think you already know everything there is to know about circulation and gas exchange in snakes.

One of the most amazing aspects of snake biology is the way they perceive the environment around them. Snakes are famous for their chemosensory abilities, and some groups of snakes are well-known for their thermal and infrared radiation detection abilities. The chapter on how snakes perceive the world covers those topics in excellent fashion. Even though hearing is less of an obvious pathway for snakes in sensing the environment, the coverage on that is well done here too. When I teach herpetology, I love to talk about snake eyes because of all the novel structures (and of the typical structures that are absent), because it seems fairly well-established that in the evolution of snakes the eyes were radically reduced if not essentially lost.



Title: *How Snakes Work: Structure, Function and Behavior of the World's Snakes*

Author: Harvey B. Lillywhite

Copyright: 2014

ISBN: 978-0-19-538037-8

Publisher: Oxford University Press

Pages: xiii + 241; **Price:** \$61 (USD)

Thus, I was disappointed to see that all those interesting things were not pointed out in the discussion of snake eyes in the book. The illustration of the eye was simplistic and sort of generic. For example, there are no muscles associated with the ciliary body in snake eyes, and the conus, sphincter, and dilatator all have mesodermal origins in snakes, but are ectodermal (!) in lizards. Those are big differences.

Snake vision received the same generic treatment. For example, regarding pupil shape, while it's true that a slit pupil can close more completely than a round pupil, which the author attributes to crepuscular and nocturnal activity, that's not the primary function. There are lots of diurnal snakes with slit pupils. The real function is that all animals with a slit pupil also have a multifocal lens. The slit allows a reduction in light while still allowing all of that light to pass through all peripheral layers of the lens (which have different refractive indices or color filters), rather than just through the lens center. No doubt, almost every aspect of snake vision is poorly known, but what is known is spectacular and its coverage in greater detail would have fit perfectly well with the rest of this excellent book.

Book Review: How Snakes Work

When people think of snakes, how many first think of sound production? Perhaps folks who mainly encounter rattlesnakes during their lives may associate snakes with sounds, but typically snakes are associated with their stealth, with their silence. And that is what makes the chapter on sound so enjoyable. **I want to hear a gopher snake bellow!** I want to hear a cloacal pop or a king cobra growl! Like vision, there is much that is not known about snake sound production and communication, and the author does a nice job bringing up those questions.

The beginning and end of lineages is intimately tied to successful reproduction, and the book closes with a chapter on the subject. It is another very well-done

chapter, with cultural and biological information as well as bits of expert-based speculation included. I greatly suspect that for many non-experts this chapter will hold a number of surprises about snakes, which really, in a nutshell, is what is great about this book. I very much like the encouraging, upbeat style of writing that gets the reader excited about the topics.

I think this is a terrific book for readers with a variety of knowledge levels. I really believe elementary school aged kids can get excited by this book and grow with the book, appreciating it more and more as they learn and progress through school. I also think the book fits well at the university level and would be a welcome addition to any biologist's library, including herpetologists!



Brian I. Crother is the Schlieder Foundation Professor of Biological Sciences and interim Department Head of Computer Sciences at Southeastern Louisiana University in Hammond, Louisiana, USA. Brian earned his B.S. from California State University at Dominguez Hills, his Ph.D. from the University of Miami, Florida, USA, and he conducted post-doctoral research at the University of Texas, Austin. He has well over 100 publications on a broad range of topics, including edited books on *Caribbean Amphibians and Reptiles*, *Ecology and Evolution in the Tropics: A Herpetological Perspective* and *Assumptions that Inhibit Progress in Comparative Biology*. Brian was the chair and coauthor of the 5th through 8th editions of the *Scientific and Standard English Names of Amphibians and Reptiles of North America North of Mexico*. He is active in several professional organizations and is an ex-president of both the *Society for the Study of Amphibians and Reptiles* and the *American Society of Ichthyologists and Herpetologists*. Brian's research interests are broad, but have in common that they cover amphibians, reptiles, and/or evolution (empirical, theoretical, and philosophical).



The herpetofauna of Coahuila, Mexico: composition, distribution, and conservation status

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Abstract.—The herpetofauna of Coahuila, Mexico, is comprised of 143 species, including 20 anurans, four caudates, 106 squamates, and 13 turtles. The number of species documented among the 10 physiographic regions recognized ranges from 38 in the Laguna de Mayrán to 91 in the Sierras y Llanuras Coahuilenses. The individual species occupy from one to 10 regions ($\bar{x} = 3.5$). The numbers of species that occupy individual regions range from 23 in the Sierras y Llanuras Coahuilenses to only one in each of three different regions. A Coefficient of Biogeographic Resemblance (CBR) matrix indicates numbers of shared species among the 10 physiographic regions ranging from 20 between Llanuras de Coahuila y Nuevo León and Gran Sierra Plegada to 45 between Serranías del Burro and Sierras y Llanuras Coahuilenses. A similarity dendrogram based on the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) reveals that the Llanuras de Coahuila y Nuevo León region is most dissimilar when compared to the other nine regions in Coahuila (48.0 % similarity); all nine other regions cluster together at 57.0% and the highest similarity is 92.0% between Laguna de Mayrán and Sierra de la Paila. The distribution patterns concerning numbers of shared species reflect higher similarity between regions that share geographic contact with each other and have comparable ecological parameters. The percentage of species restricted to one or two physiographic regions is 63.6%, indicating a moderately narrow distribution for many species. The largest number of species is placed in the non-endemic category (100), followed by the country endemics (31), state endemics (nine), and non-natives (three). The principal environmental threats to the herpetofauna are urban development, industrial pollution, deforestation, road effects, mining and energy projects, natural gas fracking, wind turbines, elimination due to cultural beliefs and practices, commercial trade, and forest fires. The conservation status of the native species is assessed by using the SEMARNAT (NOM-59), IUCN, and EVS systems, of which the EVS system proved to be the most useful. The EVS rankings also were used to determine how species in the IUCN categories of Not Evaluated (NE) and Least Concern (LC) might be evaluated more informatively. Using the Relative Herpetofaunal Priority (RHP) methodology, we determined that the most significant herpetofaunas are those of the Gran Sierra Plegada and the Sierras y Llanuras Coahuilenses. Nineteen protected areas are established in Coahuila and we predict that 119 of the 143 species in the state occur in them, based on their respective physiographic distributions. Finally, a set of conclusions and recommendations for the future protection of the Coahuilan herpetofauna is presented.

Key words. Amphibians, anurans, caudates, physiographic regions, protected areas, protection recommendation, reptiles, squamates, turtles

Resumen.—La herpetofauna de Coahuila, México consiste de 143 especies, incluyendo 20 anuros, cuatro caudados, 106 escamosos, y 13 tortugas. El número de especies documentadas entre las 10 regiones fisiográficas reconocidas va de 38 en la Laguna de Mayrán, a 91 en Sierras y Llanuras Coahuilenses. Las especies individuales ocupan de una a 10 regiones ($\bar{x} = 3.5$). El mayor número de especies en una sola región va de 23 en la Sierras y Llanuras Coahuilenses a una en cada una de las tres regiones. Una matriz de coeficiente de similitud biogeográfica (CSB) indica que el número de especies compartidas entre las 10 regiones fisiográficas va de 20 entre Llanuras de Coahuila y Nuevo León y la Gran Sierra Plegada a 45 entre Serranías del Burro y Sierras y Llanuras Coahuilenses. Un dendrograma de similitud basado en el Método por Agrupamiento de Pares no Ponderado con Media Aritmética (MAPMA) revela que sobre una base jerárquica, Llanuras de Coahuila y Nuevo León es la más desigual cuando se le compara con las otras nueve regiones en Coahuila (48.0% similitud); todas las nueve regiones se agrupan en 57.0% y la mayor similitud es 92,0% entre Laguna de Mayrán y Sierra de la Paila. Los patrones de distribución con respecto al número de especies compartidas reflejan una mayor similitud entre las regiones en contacto geográfico y con parámetros ecológicos comparables. El porcentaje

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de especies restringidas a una o dos regiones fisiográficas es de 63.6%, indicando una distribución moderada para muchas especies. El mayor número de especies está ubicado en la categoría de no endémica (100), seguido de endémicas al país (31), endémicas al estado (nueve), y no nativas (tres). Las principales amenazas ambientales a la herpetofauna son el desarrollo urbano, contaminación industrial, deforestación, efectos de carreteras, actividad minera, actividad petrolera (fracking), turbinas eólicas, matanza por falta de educación y para uso medicinal, colecta y comercio, e incendios forestales. Calculamos el estatus de conservación de las especies nativas usando los sistemas de SEMARNAT (NOM-059), UICN, y el EVS, de los cuales el EVS resultó ser más útil. También usamos los rangos de EVS para determinar cómo las especies en las categorías de No Evaluada (NE) y de Preocupación Menor (PM) de la UICN podrían ser evaluadas de una forma más informativa. Asimismo, usando el método de Prioridad Herpetofaunística Relativa (PHR), determinamos que la herpetofauna más significativa es la de Gran Sierra Plegada y la de Sierras y Llanuras Coahuilenses. Diecinueve áreas protegidas han sido establecidas en Coahuila y predecimos que 119 de las 143 especies que ocurren en el estado serán encontradas en estas áreas, basado en su distribución geográfica. Finalmente, incluimos un grupo de conclusiones y recomendaciones para la futura protección de la herpetofauna de Coahuila.

Palabras claves. Anfibios, anuros, caudados, regiones fisiográficas, áreas protegidas, recomendaciones de protección, reptiles, escamosos, tortugas

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“Like it or not, and prepared or not, we are the mind and stewards of the living world. Our own ultimate future depends upon that understanding.”

E. O. WILSON (2016)

Introduction

Coahuila is the third largest state of Mexico after Chihuahua and Sonora, all of which border the United States of America. Coahuila is bounded to the north by the US state of Texas, to the west by the Mexican states of Chihuahua and Durango, to the south by Zacatecas and a small portion of San Louis Potosí, and to the east by Nuevo León. Coahuila encompasses 151,595 km² and falls in size between the US states of Illinois and Georgia (<http://cuentame.inegi.gob.mx/monografias/informacion/coah/default.aspx?tema=me&e=05>; accessed 26 August 2017). Coahuila’s population was 2,954,915 in 2015, which is 2.5% of the same-year estimate for the entire country of Mexico (119,530,753). Coahuila is one of the six least densely populated states in Mexico (the others are Chihuahua, Sonora, Campeche, Durango, and Baja California Sur), each of which has fewer than 20 inhabitants per square kilometer; the figure for Coahuila is 19.5 (<http://cuentame.inegi.gob.mx/monografias/informacion/coah/default.aspx?tema=me&e=05>; accessed 26 August 2017). The capital and largest city in Coahuila is Saltillo, located in the southeastern portion of the state.

Much of Coahuila lies within the borders of the Chihuahuan Desert (Lazcano et al. 2017), except for the northeastern portion located within the Llanuras de

Coahuila y Nuevo León region and the southeastern corner in which is found a small portion of the Gran Sierra Plegada region (Fig. 1). In Mexico, the Chihuahuan Desert also encompasses “a large portion of the state of Chihuahua..., northeastern Durango, the extreme northern part of Zacatecas, and small western portions of Nuevo León” (<https://www.worldwildlife.org/ecoregions/na1303>; accessed 24 December 2017).

The purpose of this paper, similar to that of the others in the Mexican Conservation Series (see below), is to document the composition, physiographic distribution, and conservation status of the herpetofauna of Coahuila. In general, the format of the earlier papers in this series is followed here.

Materials and Methods

Our Taxonomic Position

In this paper, we follow the same taxonomic position as explained in previous works on other portions of Mesoamerica (Johnson et al. 2015a,b; Mata-Silva et al. 2015). Johnson (2015b) can be consulted for a statement of this position, with special reference to the subspecies concept.

Updating the Herpetofaunal List

Several recent works on the herpetofauna of Coahuila are available. Lemos-Espinal and Smith (2007) created a bilingual (Spanish and English) treatment of the state herpetofauna, in which they recognized 129 species. Eight years later, Lemos-Espinal et al. (2015) compiled

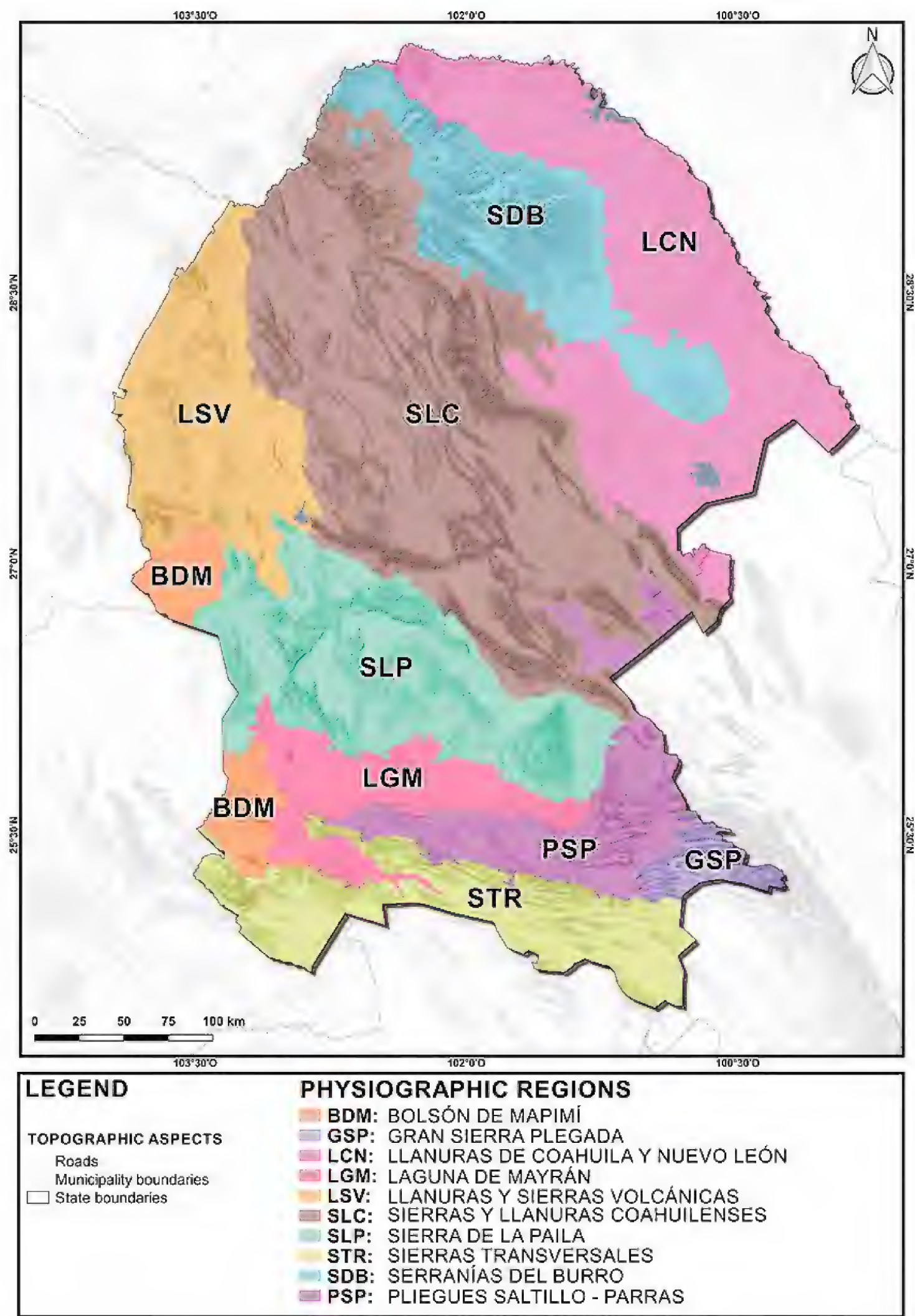


Fig. 1. Physiographic regions of Coahuila, Mexico. Abbreviations are as follows: BDM = Bolsón de Mapimi; GSP = Gran Sierra Plegada; LCN = Llanuras de Coahuila y Nuevo León; LGM = Laguna de Mayrán; LSV = Llanuras y Sierras Volcánicas; PSP = Pliegues Saltillo-Parras; SLC = Sierras y Llanuras Coahuilenses; SLP = Sierra de la Paila; STR = Sierras Transversales.

a two-volume bilingual compendium of the herpetofauna of three Mexican states (Chihuahua, Coahuila, and Sonora), in which they recorded 131 species for Coahuila. In the same year, Lemos-Espinal (2015) edited a book on the herpetofauna found in the states along the Mexico-US border, and recorded 133 species for Coahuila. Finally, Lemos-Espinal and Smith (2016) produced a checklist of the Coahuila herpetofauna, in which they again reported 133 species for the state. The name usages indicated in the Taxonomic List located at

the *Mesoamerican Herpetology* website (<http://www.mesoamericanherpetology.com>; accessed 8 March 2018) are followed here.

System for Determining Distributional Status

The system developed by Alvarado-Díaz et al. (2013) for the herpetofauna of Michoacán is employed to ascertain the distributional status of members of the herpetofauna of Coahuila. Mata-Silva et al. (2015), Johnson et al.



Fig. 2. *Bolsón de Mapimí.* Vegetation in the Bolsón de Mapimí, in the municipality of the same name in the neighboring state of Durango. Photo by Gabriel Viesca Ramos.

(2015a), Terán-Juárez et al. (2016), Woolrich-Piña et al. (2016, 2017), Nevárez-de los Reyes et al. (2016), Cruz-Sáenz et al. (2017), and González-Sánchez et al. (2017) used this system, which consists of the following four categories: SE = endemic to Coahuila; CE = endemic to Mexico; NE = not endemic to Mexico; NN = non-native in Mexico.

Systems for Determining Conservation Status

Assessment of the conservation status of the herpetofauna of Coahuila, employed the same systems (i.e., SEMARNAT, IUCN, and EVS) used by Alvarado-Díaz et al. (2013), Mata-Silva et al. (2015, 2019), Johnson et al. (2015a,b), Terán-Juárez et al. (2016), Woolrich-Piña et al. (2016, 2017), Nevárez-de los Reyes et al. (2016), Cruz-Sáenz et al. (2017), González-Sánchez et al. (2017), and DeSantis et al. (2018). Detailed descriptions of these three systems appear in earlier papers in this series.

The Mexican Conservation Series

The Mexican Conservation Series (MCS) was initiated in 2013, with a study of the herpetofauna of Michoacán (Alvarado-Díaz et al. 2013), as a part of a set of five papers designated as the Special Mexico Issue published in *Amphibian & Reptile Conservation*. The basic format of the entries in the MCS was established in that paper, i.e., to examine the composition, physiographic distribution, and conservation status of the herpetofauna of a given Mexican state or group of states. Two years later, the MCS was continued with papers on the herpetofauna of Oaxaca (Mata-Silva et al. 2015) and Chiapas (Johnson

et al. 2015a). In the ensuing year, three entries in the MCS appeared, those on Tamaulipas (Terán-Juárez et al. 2016), Nayarit (Woolrich-Piña et al. 2016), and Nuevo León (Nevárez-de los Reyes et al. 2016). Finally, three entries on Jalisco (Cruz-Sáenz et al. 2017), the Mexican Yucatan Peninsula (González-Sánchez et al. 2017), and Puebla (Woolrich-Piña et al. 2017) appeared. Thus, this paper on the herpetofauna of Coahuila is the 10th entry in this series.

Physiography and Climate

Physiographic Regions

The classification system of physiographic regions (= subprovinces) developed by INEGI in 2004 was used to analyze the distribution of the herpetofauna of Coahuila. This system consists of 10 regions (Fig. 1), which are briefly described below (see INEGI, <http://www.inegi.org.mx/est/contenidos/proyectos/ce/ce2004/presentacion.aspx>).

Bolsón de Mapimí (BDM). This region, which encompasses 4,715 km² (3.1% of the state area), is entirely confined within Mexican territory and runs along the Sierra Madre Occidental, eventually expanding to the east in the Mapimí zone. Plains and bajadas dominate the landscape, although small sierras and lomeríos facing north-south are also found there. The sierras and lomeríos located to the north are composed predominantly of volcanic rocks and are found associated with faults on their flanks; to the south limestone is the most abundant rock. The northern portion of the region is transected by



Fig. 3. *Gran Sierra Plegada*. Vegetation in the vicinity of Monterreal in the municipality of Arteaga. Photo by Elí García-Padilla.

the Río Florido and its effluents, and tributaries of the Río Conchos; the southern portion is crossed by the Río Nazas. Superficial water sources, however, are scarce. The Bolsón de Mapimí is a flat region at elevations around 1,200 m, located between Sierra del Diablo, Sierra Mojada, and irrigation district no. 17. The latter region, also known as the Comarca Lagunera or La Laguna, used to be inundated every summer by the waters of the Río Nazas until the construction of the Reservoir Francisco Zarco in the state of Durango. The arid plains of Mapimí are interrupted by low geomorphological features such as sand dunes in the northeastern portion. Deep soils of alluvial or lacustrine origin are well-represented in the plains. Most of the original vegetation around Torreón, Matamoros, and San Pedro de las Colonias has been replaced by agricultural fields. A section of the region in Laguna del Rey, however, contains microphyll desert scrub; and a similar habitat is found in the ridges of Mojada and Montaña del Rey. The middle of this region contains sand dunes, and the vegetation consists primarily of Gobernadora/Creosote Bush (*Larrea tridentata*) and huizaches (*Vachellia* [*Acacia*] spp.), providing minimum surface cover. The landscapes at La Laguna and El Guaje are represented by halophytic vegetation, and of less importance are small areas of grassland and scrubland where the main plant species is *L. tridentata*.

Llanuras y Sierras Volcánicas (LSV). This region covers approximately 14,000 km² (9.2%) of the state of Coahuila, with elevations ranging between 600 m and 1,200 m. The largest area of this territory is represented by plains or bajadas; these flat surfaces are more prominent and less

disrupted at Llano de los Ranchos, south of the mountain ranges that rise along the Río Bravo/Grande and at Bolsón de los Lipanes to the north of Sierra Mojada. This region includes small sierras of volcanic rock, such as Sierra el Mulato, Ocotillo, and Hechicero, located to the southeast of Ojinaga and the banks of the Río Bravo. Small mountains of limestone arise at the southern edge border with Bolsón de Mapimí, as in La Mojada and El Diablo. Some streams originating in this region feed the Río Bravo, and some accumulate water for short periods of time, but the climate regime is that of a desert.

The landscape in this region is dominated by shrubs, which are generally shorter than two m. Microphyll desert scrub is found with some variations in its components, with plains and slopes mostly vegetated by Gobernadora (*L. tridentata*), Viscid Acacia/Huizache (*Vachellia vernicosa*), Ocotillo (*Fouquieria splendens*), and mesquite (*Prosopis* spp.); there are also other vegetation elements, such as gatuño/Mimosa (*Mimosa* spp.), Purple Prickly-pear (*Opuntia macrocentra*), and a minor proportion of huizaches reaching heights of less than 1.5 m. The components of the lower strata are Cenizo (*Leucophyllum frutescens*), Mariola (*Parthenium incanum*), Hierba del Burro/Zinnia (*Zinnia acerosa*), and Plumed Crinklemat (*Tiquilia greggii*). Arborescent yuccas (*Yucca* spp.) and Viscid Acacia (*V. vernicosa*) also are found, reaching heights of more than four m. This community is widely distributed in the region, in all the bajadas that feed the lagoon El Guaje and the western plains.

Laguna de Mayrán (LDM). Laguna de Mayrán covers 7,804 km² (5.1%) of the state, and is mostly represented



No. 1. *Anaxyrus speciosus* (Girard, 1854). The Texas Toad is distributed from “southeastern New Mexico and western Oklahoma (USA) south throughout central and West Texas to central Tamaulipas, northern Nuevo León, northern and eastern Coahuila, and northeastern Chihuahua” in Mexico (Frost 2018). This individual came from Allende, in the municipality of Allende. Wilson et al. (2013b) calculated its EVS as 12, placing it in the upper portion of the medium vulnerability category. Its conservation status has been considered as Least Concern by IUCN, but this species is not listed by SEMARNAT. *Photo by Michael S. Price.*



No. 2. *Craugastor augusti* (Dugès, 1879). The Common Barking Frog occurs from “Arizona to Texas in the United States, and in Mexico from Sonora to Oaxaca, and from Chihuahua, Coahuila, Nuevo León, and Tamaulipas to Puebla” (Lemos-Espinal and Dixon 2013: 42). This individual was found at Cuatrociénegas in the municipality of Cuatrociénegas de Carranza. Wilson et al. (2013b) ascertained its EVS as 8, placing it in the upper portion of the low vulnerability category. Its conservation status has been evaluated as Least Concern by IUCN; this species is not listed by SEMARNAT. *Photo by Michael S. Price.*



No. 3. *Lithobates berlandieri* (Baird, 1859). The Rio Grande Leopard Frog ranges from “central and western Texas and southern New Mexico (USA) through eastern Chihuahua to central Veracruz and Hidalgo, Mexico; introduced into the lower Colorado River and lower Gila River drainages of Sonora and Baja California del Norte, Mexico, and California and Arizona, USA.” (Frost 2018). This individual was found at El Oso, in the municipality of Cuatrociénegas de Carranza. Wilson et al. (2013b) calculated its EVS as 7, placing it at the middle portion of the low vulnerability category. Its conservation status has been considered as Least Concern by IUCN, and as a species of special protection (Pr) by SEMARNAT. *Photo by Michael S. Price.*



No. 4. *Barisia ciliaris* (Smith, 1942). The Sierra Alligator Lizard is a Mexican endemic distributed “along the Sierra Madre Oriental from Nuevo León and southeastern Coahuila southward to at least Guanajuato, and northward along the Sierra Madre Occidental to extreme southern Chihuahua” (Lemos-Espinal and Dixon 2013: 97). Pictured here is an individual encountered near Monterreal, in the municipality of Arteaga. Wilson et al. (2013a) determined its EVS to be 15, placing it in the lower portion of the high vulnerability category. Its conservation status has not been assessed by IUCN, and this species is not listed by SEMARNAT. *Photo by Elí García-Padilla.*

by grassland and some hills found in the municipalities of Francisco I. Madero, General Cepeda, Parras, San Pedro, and Viesca. This region includes endorheic terminal basins of the Nazas and Aguanaval rivers. These rivers emerge in the Sierra Madre Occidental province, and flow northward through the province of Mesa del Centro to the Mayrán lagoon. The region is mostly represented by two bodies of water, Laguna de Mayrán, formerly fed by the Nazas and Viesca rivers, and Laguna de Viesca, a smaller water body fed by the Aguanaval River. Both lagoons are located at an elevation of ca. 1,400 m, with a west-east orientation, and are separated from each other by a phalanx of the Sierra Madre Oriental. Until recently, these deposits used to store a significant amount of water for most of the year, but currently their nearly level surfaces have turned into desert plains, with saline areas at the center of the Mayrán lagoon and almost the entire Viesca lagoon. Their disappearance as lakes and their final passage to a desert regime are the result of reservoirs and canal systems built on the Nazas and Aguanaval rivers for irrigation of the Laguna district; activities that, on the other hand, have significantly increased productivity in other areas.

Halophytic vegetation is the dominant element of the landscape, as the soils contain high concentrations of salts. In fact, roughly from San Pedro de las Colonias to the boundaries of the Mayrán lagoon and throughout the region, there is no other component than halophytic vegetation. The area around Mesa Albardienta is completely devoid of vegetation due to hypersaline conditions in the soil. Halophytic vegetation in this region is represented by Saladillo/Salt Bushes (*Atriplex* spp.), Seepweeds (*Suaeda* spp.), and Dropseed (*Sporobolus* spp). Besides these types of vegetation, there are small areas with microphyll desert scrub and rosette scrub in the Sierrita San Lorenzo.

Sierras y Llanuras Coahuilenses (SLC). This region covers 43,937 km² (29.0%) of the state. It includes the municipalities of Abasolo, Frontera, Lamadrid, Nadadores, Sacramento, and San Buenaventura, as well as parts of Acuña, Candela, Castaños, Cuatrociénegas, Escobedo, Monclova, Múzquiz, Ocampo, Progreso, and Ramos Arizpe, and very small portions of San Juan de Sabinas and Zaragoza. It consists of folded limestone mountains ranges, oriented northwest to southeast, mostly with steep small folds. Most of the mountains lie between elevations of 1,000 to 2,000 m, although peaks with elevations over 2,000 m can be found only in Sierra El Carmen and Sierra de San Antonio. The region has mostly internal drainage, so its runoff contributions to the Río Bravo are minimal.

Vegetation types present in the region are submontane scrub, chaparral, microphyll desert scrub, and rosette scrub. The chaparral is generally a dense shrub community, which is distributed in the transition zone between the arid scrubland and forests. In this region, it

represents an intermediate layer between the submontane shrubs and forest, and is also frequently found mixed as chaparral and desert scrub. It is composed of shrubby oaks (*Quercus* spp.) and shrub species in the genera *Cercocarpus* and *Vauquelinia*, among others. Other shrub components such as sotols (*Dasyllirion* spp.) and yuccas (*Yucca* spp.) are also present.

Serranías del Burro (SDB). This region encompasses 13,234 km² (8.7%) of the state, and includes parts of the municipalities of Acuña, Guerrero, Múzquiz, Sabinas, Villa Unión, and Zaragoza, as well as very small portions of Juárez, Morelos, and San Juan de Sabinas. The Serranías del Burro has a normal fault on its northwestern flank. It is rugged in its central part, which includes a radial system of narrow valleys, but lies much more towards the east and southeast, where the sierra becomes narrow and descends into the hilly zone of Peyotes. A series of igneous intrusions crosses the Serranías del Burro from east to west near Villa Acuña and Cerro El Colorado, with the latter representing the highest peak of the mountain range, with an elevation of 1,400 m. The region has few major streams, although it has slopes toward the Río Bravo. Soils in the topoform systems (sierras) constituting this region are mainly represented by shallow lithosols, while xerosols, phaeozems, and regosols cover the small systems of hills (lomeríos). Soils on slopes (bajadas) have colluvial or colluvial-alluvial sources, and the edaphic landscapes in the intermontane valleys of the Serranías del Burro are very similar to those in the other regions mentioned above. The vegetation types found in this region are typical of the arid zones of Mexico. The hilly zones (lomeríos) that border the mountains of Colorado, del Burro, and La Babia are covered with rosette scrub. Among the main components of this vegetation are Lechuguilla (*Agave lechuguilla*), Texas Sotol (*Dasyllirion texanum*), and short yuccas (*Yucca* spp.). Submontane shrubs on the Serranías del Burro also ascend the eastern slopes of the range, with the most distinctive components being Cenizo/Purple Sage (*Leucophyllum frutescens*), Tenazas (*Havardia pallens*), Hoja Ancha/Tarbrush (*Flourensia laurifolia*), and Coyotillo/Buckthorn (*Karwinskia* sp.). Another vegetation formation of great importance in the region is chaparral, which grows below the oak forest on the Sierras del Burro, del Carmen, and La Babia; this vegetation also grows on the small hills located immediately to the north of these mountain ranges. There is also a large number of pine forests in this region, numerically dominated by Pino Piñonero (*Pinus cembroides*). Additionally, there are large extensions of grassland in the intermontane valleys immediately below the chaparral.

Finally, in the lower portion of the sierras, adjacent to the Great Plains of North America, there is Tamaulipecan thorn scrub. This vegetation is located on the hills to the south of the region bordering the rosette scrub

found on the hills of Peyotes. The main components of this vegetation formation are Palo Verde (*Cercidium texanum*), Chaparro Amargoso/Indian Paintbrush (*Castela texana*), Cenizo (*Leucophyllum frutescens*), and mesquite trees (*Prosopis* spp.).

Sierra de la Paila (SLP). This region includes sierras, large bolsons with internal drainage, and bajadas. It covers ca. 19,230 km² (12.7%) of the state. Valle Buenavista bolson is located in the western portion, bordered to the west by Sierra de Tlahualilo and to the east by the highlands of Albardienta, which reach an elevation of 1,800 m. Sierra de La Paila is located to the east, and the bolsons El Sobaco, El Hundido, San Marcos, and Los Pinos are located to the north, with the first three at less than 1,000 m elevation. As in the other regions, the soils and biodiversity in the Sierra de La Paila is influenced by climate, which is semiarid at high elevations and very arid in the grasslands and bolsons. Although topographically rugged, the sierras have relatively small portions covered with soils. There are, however, deep soils of primarily alluvial origin in the lower sections of the bolsons that have high concentrations of salts. Also, there are sandy soils of eolic origin that form dunes.

The vegetation communities in this region are mostly the same as those in Sierras Transversales and Pliegues Saltillo-Parras. Microphyllous and rosette scrub is closely associated with the terrain, and dry and semidry climatic conditions are present in the bajadas and valleys. Rosette scrub is widely distributed in all sierras such as La Fragua, La Mesa Albardienta, and La Paila. Chaparral is a community of shrubs primarily represented by oaks (*Quercus* spp.), Chapote/Texas Persimmon (*Diospyros texana*), and some elements of rosette scrub, like yuccas, Sotol, and Lechuguilla. Submontane shrub is a community composed of shrubs, primarily Chapote (*D. texana*), Tenazas (*H. pallens*), Guajillo (*Acacia berlandieri*), and Chaparro Prieto (*Acacia amentacea*). These shrubs are also found in La Paila and La Fragua at the same elevations as chaparral.

Pliegues Saltillo–Parras (PSP). This region covers 9,195 km² (6.1%) of the state of Coahuila. The landscape is represented by a set of valleys extending from east to west, situated at an elevation of approximately 1,600 m and bordered to the north and south by eroded flanks and valleys. The region also includes the Sierra de Parras, on which peaks can reach more than 3,000 m, and includes a succession of truncated large flanks toward the south. This region in Coahuila includes parts of the municipalities of Parras, Cepeda, Saltillo, Arteaga, Ramos Arizpe, Castaños, Candela, and Monclova.

Microphyll desert scrub and rosette scrub are the dominant vegetation types in this area. Rosette scrub is distributed on mountain ranges, slopes, and hills, especially in shallow soils, and alternating with microphyll scrub in flatter areas, in deep and alluvial soils.

To the north of San Martín de las Varas, rosette scrub contains some representatives of *Pinus cembroides*, but they do not modify the physiognomy of this vegetation community. Pine forests are also found in the southern part of the region, on the bajadas of the Sierra El Jabalí, where their density increases with elevation. Between the scrub and forest, there are two types of natural and introduced grassland areas. The first area is located south of General Cepeda, and to the south of Saltillo on the hills next to Estación Agua Nueva, and contains grasses of the genera *Bouteloua* and *Sporobolus*. Introduced grasslands are found to the east of Saltillo, through a substantial geographic extension of grasses belonging to the genera *Bouteloua* and *Aristida*.

Sierras Transversales (STR). This region extends throughout the southern section of the state, in the municipalities of Cuatro Ciénegas, Ocampo, and Sierra Mojada, encompassing a surface area of 14,077 km² (9.3% of the state area). Over half of this region consists of sierras with shallow light-colored soils. The main vegetation is represented by rosette scrub and microphyll desert scrubland. Rosette scrub vegetation is distributed on all the mountains, slopes, and small hills located at elevations between 2,000 and 2,400 m, with large portions of this vegetation located on the sierras El Número, Candelaria, Parras, and other smaller sierras. The main components of this vegetation are Huizache (*Acacia farnesiana*), Chapotes (*D. texana*), Texas Sotol (*D. texanum*), Lechuguilla (*A. lechuguilla*), and Gatuño (*Acacia roemeriana*). In the lower parts of the sierras Playa Madero and del Laurel, the same type of vegetation is also found, where the numerically dominant *Yucca thompsoniana* gives the appearance of an Izotal (Yucca tree forest), in addition to Sotol, Lechuguilla, Chaparro Prieto (*Acacia rigidula*), and Fresno (*Fraxinus greggii*), among others. This association also is present on the bajadas of the sierras.

The abundance of microphyll shrubs is noteworthy on flat areas, especially in the southeastern part of the state, with thorny elements present, such as mesquites (*Prosopis* spp.) and huizaches (*Acacia* spp.), as well as Gobernadora (*L. tridentata*) and Hojasén (*Flourensia cernua*). Additionally, relatively small areas of grassland are found on the sierras of Gómez Farías and Jabalí, with species of the genera *Bouteloua*, *Muhlenbergia*, *Andropogon*, and *Aristida*. The southeastern section of the state also has flat areas with halophytic vegetation on saline soils, with species of saladillo (*Suaeda* spp.) and saltbushes (*Atriplex* spp.). On the other hand, chaparral, pine-oak forest, pine forest, and small areas with submontane scrub are found in the less arid sierras, such as the Sierras de Jimulco, Parras, and Jabalí. Among components of the chaparral are small oaks (*Quercus* spp.) and Pino Piñonero (*P. cembroides*).

Gran Sierra Plegada (GSP). This region covers 2,178 km² (1.4%) of the state. It includes a major portion of the

The herpetofauna of Coahuila, Mexico

Table 1. Monthly minimum, mean (in parentheses), maximum, and annual temperature data (in °C) for the physiographic regions of Coahuila, Mexico. The locality and elevation for each region are: Bolsón de Mapimí—Laboratorio del Desierto, Tlahualilo, Durango (1,160 m); Llanuras y Sierras Volcánicas—Sierra Mojada (1,256 m); Laguna de Mayrán—Viesca (1,100 m); Sierras y Llanuras Coahuilenses—San Francisco Nadadores (500 m); Serranía del Burro—Agua Nueva (370 m); Sierra La Paila—Hipólito (1,150 m); Pliegues Saltillo-Parras—General Cepeda (1,400 m); Sierras Transversales—La Ventura (1,867 m); Gran Sierra Plegada—San Antonio de las Alazanas (2,300 m); and Llanuras de Coahuila y Nuevo León—Presa Venustiano Carranza (272 m). Data from: <http://www.smn1.conagua.gob.mx/climatologia/normales/estacion/EstacionesClimatologicas.kmz>; accessed 11 November 2017.

Physiographic Region	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Set	Oct	Nov	Dec	Annual
Bolsón de Mapimí	3.0	5.0	8.2	12.4	16.5	19.1	19.2	18.6	16.7	12.7	7.3	3.3	11.8
	(11.7)	(14.0)	(17.4)	(21.5)	(25.3)	(27.2)	(26.5)	(25.8)	(23.8)	(20.6)	(15.8)	(12.0)	(20.1)
	20.3	23.0	26.7	30.6	34.1	35.3	33.9	33.0	31.0	28.5	24.2	20.6	28.4
Llanuras y Sierras Volcánicas	4.3	5.5	8.2	12.0	15.0	16.7	16.4	16.0	14.1	11.0	7.0	4.9	10.9
	(10.6)	(12.2)	(15.6)	(19.5)	(22.6)	(24.3)	(23.5)	(22.9)	(20.8)	(17.9)	(14.1)	(11.1)	(17.9)
	16.8	18.9	23.0	27.0	30.3	31.8	30.5	29.7	27.5	24.9	21.1	17.4	24.9
Laguna de Mayrán	4.0	6.2	9.0	13.0	16.7	19.5	20.2	19.5	18.0	13.4	7.7	4.6	12.7
	(14.0)	(16.4)	(19.6)	(23.3)	(26.7)	(28.3)	(28.1)	(27.6)	(26.0)	(22.4)	(17.8)	(14.4)	(22.1)
	24.0	26.7	30.3	33.6	36.6	37.1	36.0	35.7	33.9	31.5	27.8	24.2	31.5
Sierras y Llanuras Coahuilenses	2.8	4.7	7.5	11.5	16.3	19.2	19.2	20.1	17.0	12.5	7.3	4.1	11.9
	(11.0)	(14.0)	(17.4)	(21.5)	(25.7)	(28.0)	(27.8)	(28.3)	(24.8)	(20.7)	(15.8)	(12.1)	(20.6)
	19.3	23.3	27.2	31.6	35.2	36.7	36.3	36.5	32.5	28.8	24.3	20.2	29.3
Serranía del Burro	4.6	5.0	7.5	12.3	15.7	18.4	18.8	18.9	16.3	12.1	7.7	4.4	11.8
	(11.7)	(12.7)	(15.8)	(20.7)	(23.8)	(25.8)	(25.8)	(25.1)	(22.8)	(18.6)	(14.5)	(10.6)	(19.0)
	18.9	20.5	24.0	29.2	31.9	33.3	32.8	31.3	29.2	25.1	21.2	16.7	26.2
Sierra La Paila	9.6	10.7	12.8	14.5	17.6	19.5	19.5	19.4	17.6	15.9	13.1	11.1	15.1
	(15.0)	(16.0)	(17.9)	(20.2)	(23.0)	(25.1)	(25.2)	(24.8)	(22.6)	(21.0)	(18.4)	(16.1)	(20.4)
	20.3	21.3	23.1	25.9	28.3	30.8	30.9	30.2	27.6	26.1	23.6	21.1	25.8
Pliegues Saltillo-Parras	5.3	6.5	9.4	12.9	15.8	17.5	17.4	16.9	15.0	12.2	8.4	6.2	12.0
	(12.8)	(14.4)	(17.9)	(21.2)	(24.0)	(25.0)	(24.6)	(24.0)	(21.8)	(19.5)	(15.9)	(13.5)	(19.6)
	20.2	22.3	26.3	29.5	32.1	32.5	31.8	31.0	28.6	26.7	23.4	20.8	27.1
Sierras Transversales	1.9	3.1	5.3	7.5	10.9	12.3	13.3	12.6	12.2	8.8	5.6	2.1	8.0
	(11.7)	(13.1)	(16.0)	(18.5)	(21.6)	(22.5)	(22.3)	(21.7)	(21.5)	(19.4)	(15.5)	(12.0)	(18.0)
	21.5	23.1	26.8	29.4	32.3	32.6	31.3	30.8	30.8	29.9	25.5	21.9	28.0
Gran Sierra Plegada	4.2	5.0	6.2	7.8	9.0	9.5	9.6	9.1	8.6	7.2	5.6	4.6	7.2
	(12.2)	(13.2)	(14.6)	(16.4)	(17.5)	(17.5)	(17.3)	(16.9)	(16.6)	(15.6)	(14.2)	(12.7)	(15.4)
	20.2	21.3	22.9	25.1	25.9	25.5	24.9	24.4	24.6	23.7	22.9	20.7	23.5
Llanuras de Coahuila y Nuevo León	4.9	7.0	10.7	15.1	19.0	21.7	22.6	22.5	20.3	15.7	9.8	5.6	14.6
	(11.9)	(14.5)	(18.5)	(22.8)	(26.0)	(28.7)	(29.5)	(29.3)	(26.7)	(22.3)	(16.8)	(12.8)	(21.7)
	18.9	22.0	26.2	30.5	33.1	35.7	36.5	36.1	33.1	28.9	23.8	19.9	28.7

municipality of Arteaga (95%) and a very small fraction of Saltillo. This region begins east of Saltillo, Coahuila, but also includes sections in Nuevo León, Tamaulipas, and San Luis Potosí, and is dominated by folded layers of limestone. A great reverse geological fault lies on the eastern edges of the Gran Sierra Plegada, while smaller ones extend relatively parallel to it and its structural axes. The elevational range in the region is between 2,000 and 3,750 m.

The topography is predominantly mountainous, but also contains plateaus and valleys. This region is located somewhat parallel to the Gulf of Mexico and represents an orographic barrier that favors the deposition of moisture on the eastern slopes, which prevents the westward movement of moist winds. Heavy rainfall has led to the dissolution of limestone rocks in the area, resulting in a karstic environment. These processes have led to the formation of vast cavern systems and springs at the foot of the mountains. A broad elevational gradient is present in this area. The soils are dominated by lithosols, which are associated with rendzinas and calcaric regosols.

Calcic and haplic xerosols are also found within the region.

In general, two fundamental forms of plant landscapes are present in the region: forests and scrublands. Pines dominate the forested area, and desert rosette scrub, piedmont scrub, and chaparral dominate the rest of the region. Other types of vegetation in the Gran Sierra Plegada occur as small patches of grassland, halophytic vegetation, or alpine prairie, but they have minimal influence in shaping the overall landscape.

Llanuras de Coahuila y Nuevo León (LCN). This region encompasses 25,666 km² (16.9% of the state area), including the municipalities of Hidalgo, Nava, Piedras Negras, and Jiménez, and parts of Guerrero, Villa Unión, Morelos, Allende, Progreso, Escobedo, Sabinas, San Juan de Sabinas, Nueva Rosita, Múzquiz, Zaragoza, and Acuña. The region is characterized by the presence of plains interrupted with scattered low hills that are composed of conglomerates, at elevations ranging from



Fig. 4. *Llanuras de Coahuila y Nuevo Leon*. Tamaulipas thorn scrub in the municipality of Allende. Photo by Manuel Nevárez de los Reyes.



Fig. 5. *Laguna de Mayrán*. Vegetation on Cerro de la Virgen, in the municipality of Parras. Photo by José Flores Ventura.



Fig. 6. *Llanuras y Sierras Volcánicas*. Vegetation near Hércules, in the municipality of Sierra Mojada. Photo by Daniel Solorio Estrada.



Fig. 7. *Pliegues Saltillo-Parras*. Vegetation near Parras de la Fuente, in the municipality of Parras. Photo by Manuel Nevárez de los Reyes.

75 to about 500 m. One of the most extensive plains extends from Anahuac, Nuevo León, to Nueva Rosita, Coahuila, at an average elevation of 500 m.

Tamaulipan thornscrub and mesquites (*Prosopis* spp.) are the most characteristic vegetation types in this region. Tamaulipan thornscrub is distributed at elevations from 80 to 340 m, with a physiognomy of thornscrub in areas of low relief and of semi-thorn scrubland on the lower sections of areas with higher relief. Large patches of Cenizo (*L. frutescens*) are present in some areas, indicative of a high degree of disturbance to the native scrub vegetation, as this species numerically dominates the sympatric native species that are found in low frequency and are small in size. Mesquites dominate at elevations from 75 to 400 m. Piedmont scrub or Tamaulipan thornscrub predominate in some middle sections, and a prevalence of halophytic elements is present in the lower areas. Some deciduous thornscrub and deciduous hardwood forests are found in the region as well, and oak and pine-oak forests occur at higher elevations. Halophytic vegetation is found within small areas of the plains and valleys, where high salt concentrations are present in the soils. Natural grassland

occurs in some areas of the plains at elevations from 135 to 290 m. The introduced grasses on the plains and valleys are composed primarily of Buffelgrass (*Pennisetum ciliare*), which is distributed at elevations from 190 to 270 m and covers small hilly areas and alluvial plains.

Climate

Temperature. The minimum, mean, and maximum temperatures for one locality in each of the 10 physiographic regions in Coahuila are shown in Table 1. The elevations for these 10 regions range from 272 m in the Llanuras de Coahuila y Nuevo León to 2,300 m in the Gran Sierra Plegada.

The mean annual temperature (MAT) of these regions ranges from a low of 15.4 °C, in the Gran Sierra Plegada at 2,300 m in the southeastern portion of the state, to a high of 22.1 °C, in the Laguna de Mayrán at 1,100 m in the southern portion of the state. The MAT of these regions in Coahuila are unusual in that they do not gradually decrease with increasing elevation. The MAT lie below 20 °C in four additional regions, including the Llanuras y Sierras

Table 2. Monthly and annual precipitation data (in mm) for the physiographic regions of Coahuila, Mexico. The locality and elevation for each region are: Bolsón de Mapimí—Laboratorio del Desierto, Tlahualilo, Durango (1,160 m); Llanuras y Sierras Volcánicas—Sierra Mojada (1,256 m); Laguna de Mayrán—Viesca (1,100 m); Sierras y Llanuras Coahuilenses—San Francisco Nadadores (500 m); Serranía del Burro—Agua Nueva (370 m); Sierra La Paila—Hipólito (1,150 m); Pliegues Saltillo-Parras—General Cepeda (1,400 m); Sierras Transversales—La Ventura (1,867 m); Gran Sierra Plegada—San Antonio de las Alazanas (2,300 m); Sierras y Llanuras Occidentales—Carbonera, Galeana, Nuevo León (2,035 m); and Llanuras de Coahuila y Nuevo León—Presa Venustiano Carranza (272 m). The shaded area indicates the months of the rainy season. Data from: <http://www.smn1.conagua.gob.mx/climatologia/normales/estacion/EstacionesClimatologicas.kmz>; accessed 11 November 2017.

Physiographic Region	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Annual
Bolsón de Mapimí	9.5	4.0	4.2	8.9	15.2	36.2	46.6	54.8	42.4	20.8	9.1	6.9	258.6
Llanuras y Sierras Volcánicas	14.7	6.5	4.8	9.4	23.8	50.0	66.1	68.9	63.5	24.7	11.8	12.1	356.3
Laguna de Mayrán	11.4	3.0	2.5	4.1	7.1	19.3	24.9	27.1	28.5	13.4	8.1	10.5	159.9
Sierras y Llanuras Coahuilenses	10.4	6.4	7.3	13.9	35.2	38.1	49.6	36.5	58.1	28.3	10.1	14.9	308.8
Serranías del Burro	8.1	3.5	2.2	2.3	4.1	11.8	11.1	21.6	17.0	9.2	2.0	3.8	96.7
Sierra La Paila	9.0	9.1	5.8	8.2	16.8	18.0	29.1	22.3	17.7	12.7	6.8	8.6	164.1
Pliegues Saltillo-Parras	10.6	10.0	6.2	11.2	22.1	47.1	62.0	68.4	57.4	25.2	10.5	11.9	342.6
Sierras Transversales	14.8	12.8	9.3	22.8	37.4	55.9	41.8	46.9	46.9	38.7	10.5	9.1	346.9
Gran Sierra Plegada	17.5	12.4	8.4	19.7	32.8	41.2	66.3	63.2	54.1	29.8	14.7	15.3	375.4
Llanuras de Coahuila y Nuevo León	15.1	20.1	14.7	25.8	62.3	39.7	28.0	44.7	67.8	38.1	15.2	12.8	384.3

Volcánicas at 1,256 m (17.9 °C), the Serranías del Burro at 370 m (19.0 °C), the Pliegues Saltillo-Parras at 1,400 m (19.6 °C), and the Sierra Transversales at 1,867 m (18.0°C). In contrast, the MAT in the remaining five regions lie above 20 °C, including the Bolsón de Mapimí at 1,160 m (20.1 °C), the Laguna de Mayrán at 1,100 m (22.1°C), the Sierra de Paila at 1,150 m (20.4 °C), the Sierras y Llanuras Coahuilenses at 500 m (20.6 °C), and the Llanuras de Coahuila y Nuevo León at 272 m (21.7 °C).

The minimum annual temperature ranges from 7.2 °C in the Gran Sierra Plegada to 15.1 °C in the Sierra La Paila (Table 1). The maximum annual temperature varies from 23.5 °C in the Gran Sierra Plegada to 31.5 °C in the Laguna de Mayrán. The minimum annual temperature is 10.7–20.0 °C lower than the maximum annual temperature among the 10 physiographic regions of the state (Table 1). Mean monthly temperatures peak at some point from May to August, usually June, and reach a low point sometime during December or January, usually January (Table 1).

Precipitation. As expected, precipitation in Coahuila is generally highest during the rainy season from June to October, and lowest from November to May, during the dry season. The data in Table 2 indicate that 56.8–77.6% ($\bar{x}$ = 69.4%) of the rainfall occurs during the rainy season.

Table 3. Composition of the native and non-native herpetofauna of Coahuila, Mexico.

Orders	Families	Genera	Species
Anura	7	13	20
Caudata	2	3	4
Subtotals	9	16	24
Squamata	15	44	106
Testudines	4	6	13
Subtotals	19	50	119
Totals	28	66	143

The month with the greatest amount of precipitation, depending on the location, is June, July, August, or September, usually August (Table 2). The month with the least amount of precipitation, again depending on the location, is December, February, or March, usually March (Table 2). The annual precipitation ranges from 96.9 mm in the Serranías del Burro to 384.3 mm in the Llanuras de Coahuila y Nuevo León.

Composition of the Herpetofauna

Families

The herpetofaunal species of Coahuila are placed in 28 families, including seven for anurans, two for salamanders, 15 for squamates (one of which contains only a single non-native species), and four for turtles (Table 3). The seven anuran families comprise 63.6% of the 11 families with representatives in Mexico. The two salamander families constitute 50.0% of the four families represented in the country. The fifteen squamate families make up 48.4% of the 31 Mexican families containing native species. Finally, the four Coahuilan turtle families encompass 40.0% of the 10 families in Mexico. The total of 28 families includes 47.5% of the 59 herpetofaunal families that are represented in this country (Johnson et al. 2017). There are no caecilian or crocodylian families with representatives in Coahuila.

Genera

Sixty-six herpetofaunal genera are represented in Coahuila, including 13 for anurans, three for salamanders, 44 for squamates, and six for turtles (Table 3). The 13 anuran genera constitute 35.1% of the 37 with representatives in Mexico. The three salamander genera make up 15.8% of the 19 found in Mexico. Of the



No. 5. *Gerrhonotus infernalis* Baird, 1859. The Texas Alligator Lizard ranges “from central Texas westward to the area of Big Bend, in the United States, and in Mexico east of the Sierra Madre Oriental to southern San Luis Potosí and perhaps extreme southeastern Durango” (Lemos- Espinal and Dixon 2013: 98). This individual was found in Sierra La Concordia, in the municipality of General Cepeda. Wilson et al. (2013a) calculated its EVS as 13, placing it at the upper end of the medium vulnerability category. Its conservation status has been gauged as Least Concern by IUCN, and this species is not listed by SEMARNAT. *Photo by Michael S. Price.*



No. 6. *Gerrhonotus lugoi* McCoy, 1970. Lugo's Alligator Lizard is a Mexican endemic species restricted to the Cuatro Ciénegas region (Lemos-Espinal et al. 2015). This individual was found at Cuatrociénegas, in the municipality of Cuatrociénegas de Carranza. Wilson et al. (2013a) judged its EVS as 17, placing it in the middle portion of the high vulnerability category. Its conservation status is evaluated as Least Concern by IUCN, and as threatened (A) by SEMARNAT. *Photo by Michael S. Price.*



No. 7. *Gerrhonotus mccoysi* García-Vázquez, Contreras-Arquieta, Trujano-Ortega, and Nieto-Montes de Oca, 2018. McCoy's Alligator Lizard is a Mexican endemic species restricted to the Cuatro Ciénegas region (García-Vázquez et al. 2018). This individual, a male paratype of the species, was encountered at Pozas Azules, Rancho Pronatura in the municipality of Cuatro Ciénegas. The EVS of this species can be calculated as $6+8+3=17$, placing it in the middle portion of the high vulnerability category. Its conservation status has not been evaluated by IUCN, and this species is not listed by SEMARNAT. *Photo by Uri García-Vázquez.*



No. 8. *Crotaphytus antiquus* Axtell and Webb, 1995. The Venerable Collared Lizard occurs from “the Sierras de San Lorenzo, Texas and Solís in extreme western Coahuila” (Lemos-Espinal et al. 2015: 173). This individual was encountered at ca. 8 km SW from the locality of San Antonio del Coyote, in the municipality of Matamoros. Wilson et al. (2013a) determined its EVS as 16, placing it in the middle portion of the high vulnerability category. Its conservation status has been evaluated as Endangered by IUCN, but it has not been listed by SEMARNAT. *Photo by Marco Antonio Bazán-Tellez.*



Fig. 8. *Serranías del Burro*. Panoramic view of the Serranías del Burro, in the municipality of Zaragoza. Photo by Daniel Garza-Tobón.

138 genera of squamates in Mexico, the 44 represented in Coahuila amount to 31.9%. The six turtle genera comprise 33.3% of the 18 genera with representatives in Mexico. The total of 66 genera encompasses 30.6% of the 216 found in Mexico (Johnson et al. 2017).

Species

The herpetofauna of Coahuila represents 143 species, including 20 anurans, four salamanders, 106 squamates, and 13 turtles (Table 3). The 20 anuran species comprise 8.1% of the 247 distributed in Mexico. The four salamander species constitute 2.6% of the 151 found in Mexico. The 106 squamate species make up 12.3% of the 863 located in Mexico. The 13 turtle species amount to 25.5% of the 51 species occurring in Mexico. The total of 143 species comprises 10.8% of the 1,318 species making up the Mexican herpetofauna (Johnson, unpub.).

Comments on the Species List

Some comments on our list of recognized species are necessary, especially as compared to that in Lemos-Espinal and Smith (2016), as follows:

***Eleutherodactylus marnockii*.** Although Lemos-Espinal and Smith (2016) listed this frog as a resident of Coahuila, they provided no evidence based on voucher specimens. In addition, neither Dodd (2013) nor Frost (2018) list this species as occurring in Mexico. Thus, we do not include this species in our analysis.

***Ambystoma velasci*.** Although Lemos-Espinal and Smith (2016) reported *A. mavortium* in Coahuila, we consider populations of *Ambystoma* in southeastern Coahuila to belong to the same species that occurs in nearby Nuevo León, which was identified as *A. velasci* by Nevárez-de los Reyes et al. (2016).

***Gerrhonotus mccoysi*.** Recently, García-Vázquez et al. (2018) described this species from the Cuatro Ciénegas Basin in the Sierras y Llanuras Coahuilenses region.

***Gerrhonotus parvus*.** Until recently, this species was considered endemic to Nuevo León (Lemos-Espinal et al. 2016). Banda-Leal et al. (2018), however, reported it from Sierra de Zapalinamé, Coahuila. Therefore, this species presumably has a continuous distribution throughout the Gran Sierra Plegada region between the type locality (Galeana, Nuevo León) and Sierra Zapalinamé, Coahuila.

***Sceloporus bimaculosus*.** A former subspecies of *S. magister* (elevated by Schulte et al. 2006) was returned to the synonymy of *S. magister* by Leaché and Mulcahy (2007). Curiously, a few sources since then (including Lemos-Espinal and Smith's (2016) most recent checklist on the herpetofauna of Coahuila and the Reptile Database website) referenced the former publication and continued to recognize *S. bimaculosus* as a full species despite a short discussion of that issue by Wilson et al. (2015). In any event, at this point, we herein recognize *S. magister* as the species

of the *S. magister* species group occurring in Coahuila.

***Sceloporus cowlesi*.** Lemos-Espinal and Smith (2016) in their recent checklist reported *Sceloporus consobrinus* for the Coahuila herpetofauna. In a study of the molecular phylogenetics of the *Sceloporus undulatus* species group, however, Leaché (2009) restricted the distribution of *S. consobrinus* to the United States and indicated the member of the *undulatus* group found within Nuevo León to be *S. cowlesi*, which is the name we use here for the Coahuilan populations.

***Sceloporus gadsdeni*.** Díaz-Cárdenas et al. (2017) recently described this species from Sierra de San Lorenzo, near Torreón, in the Bolsón de Mapimí region.

***Sceloporus ornatus*.** Herein we regard *Sceloporus oberon* as a synonym of *Sceloporus ornatus*, based on Martínez-Méndez and Méndez-de la Cruz (2007).

***Sceloporus marmoratus*.** Unlike Lemos-Espinal and Smith (2016), we do not list *S. variabilis* as occurring in Coahuila, but based on Mendoza-Quijano et al. (1998) we do recognize *S. marmoratus* as occurring in the Llanuras de Coahuila y Nuevo León region of Coahuila, instead of *S. variabilis*.

***Lampropeltis annulata*.** *Lampropeltis annulata* was considered an evolutionary species separate from *L. triangulum* by Ruane et al. (2014), who elevated a number of subspecies of *L. triangulum* to full species and synonymized others. Subspecies synonymized with *L. annulata* included *L. t. dixonii*.

***Lampropeltis gentilis*.** The first record for this species from Mexico and Coahuila was reported by Baeza-Tarín et al. (2018a).

***Lampropeltis splendida*.** *Lampropeltis splendida* was elevated to a full species separate from *L. getula* by Pyron and Burbrink (2009).

***Salvadora deserticola*.** Nevárez de los Reyes et al. (2018) first reported this species from Coahuila.

***Tantilla cucullata*.** Baeza-Tarín et al. (2018b) first reported this species for Coahuila.

***Trimorphodon wilkinsonii*.** Baeza-Tarín et al. (2018c) first reported this species for Coahuila.

***Crotalus ornatus*.** Anderson and Greenbaum (2012) resurrected *Crotalus ornatus* from the synonymy of *C. molossus*. *Crotalus ornatus* is found in most parts of Coahuila, whereas *C. molossus* is restricted to the extreme southern parts of the state.

***Apalone atra*.** *Apalone atra* has been either regarded as a subspecies of *A. spinifera* or as a species endemic to the Valley of Cuatro Ciénegas, where *A. spinifera* has gained access to some areas through irrigation channels, thereby allowing some genetic introgression to take place, and driving *A. atra* to a level of being critically susceptible to extinction. Smith and Smith (1979) had already considered *A. atra* to be extinct. Recently, however, pure individuals of *A. atra* have been found, as well as hybrids between the two species (Cerdá-Ardura et al. 2008). See Wilson and Johnson (2010) for a discussion on this issue. Until updated data indicate otherwise, we regard *A. atra* as having viable populations in the Valley of Cuatro Ciénegas, highlighting its need for conservation assessment and action.

Patterns of Physiographic Distribution

Herein 10 physiographic regions are recognized in Coahuila (Fig. 1), and the occurrence of the members of the herpetofauna among these 10 regions are shown in Table 4 and summarized in Table 5.

The total number of species in each region ranges from a low of 38 in the Laguna de Mayrán to a high of 91 in the Sierras y Llanuras Coahuilenses (Table 5). The number of species in each of the other regions is as follows, in ascending order: 40 (Sierra de la Paila); 44 (Llanuras y Sierras Volcánicas); 45 (Bolsón de Mapimí); 45 (Serranías del Burro); 47 (Sierra Transversales); 49 (Pliegues Saltillo Parras); 51 (Gran Sierra Plegada); and 53 (Llanuras de Coahuila y Nuevo León). The lowest value of 38 in the Laguna de Mayrán is 41.8% of the highest value of 91 in the Sierras y Llanuras Coahuilenses. The latter region is the largest in the state, but the former region is not the smallest (the smallest region is the Gran Sierra Plegada).

As expected, the largest absolute and relative numbers of the component herpetofaunal groups are found in the Sierras y Llanuras Coahuilenses, including 14 of 24 species of amphibians (58.3%), 68 of 106 species of squamates (64.2%), and nine of 13 species of turtles (69.2%).

Members of the Coahuilan herpetofauna inhabit from one to all 10 of the 10 physiographic regions, as follows: one (66 of 143 species: 46.2%); two (25; 17.5%); three (11; 7.7%); four (five; 3.5%); five (0; 0%); six (0; 0%); seven (two; 1.4%); eight (five; 3.5%); nine (11; 7.7%); and 10 (18; 12.6%). The most broadly distributed species (occupying all 10 regions) are the anurans *Anaxyrus debilis*, *A. punctatus*, *Lithobates berlandieri*, *Scaphiopus couchii*, and *Spea multiplicata*; the lizards *Crotaphytus collaris*, *Coleonyx brevis*, *Hemidactylus turcicus* (non-native), *Phrynosoma cornutum*, *Sceloporus grammicus*, *S. poinsetti*, and *Aspidoscelis gularis*; and the snakes *Lampropeltis splendida*, *Masticophis flagellum*, *Pantherophis emoryi*, *Pituophis catenifer*, *Rhinocheilus lecontei*, and *Thamnophis marcianus*. Given that Coahuila

Table 4. Distribution of the amphibians, squamates, and turtles of Coahuila, Mexico, by physiographic region. Abbreviations are as follows: BDM = Bolsón de Mapimi; LSV = Llanuras y Sierras Volcánicas; LDM = Laguna de Mayrán; SLC = Sierras y Llanuras Coahuilenses; SDB = Serranías del Burro; SLP = Sierra de la Paila; PSP = Pliegues Saltillo Parras; STR = Sierras Transversales; GSP = Gran Sierra Plegada; and LCN = Llanuras de Coahuila y Nuevo León. * = species endemic to Mexico; ** = species endemic to Coahuila; and *** = non-native species. See text for detailed descriptions of these regions.

Taxa	Physiographic Regions of Coahuila										Number of Regions Occupied
	BDM	LSV	LDM	SLC	SDB	SLP	PSP	STR	GSP	LCN	
Bufonidae (7 species)											
<i>Anaxyrus cognatus</i>	+	+	+	+	+	+	+				7
<i>Anaxyrus debilis</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Anaxyrus punctatus</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Anaxyrus speciosus</i>				+	+	+	+			+	5
<i>Anaxyrus woodhousii</i>	+	+									2
<i>Rhinella horribilis</i>				+						+	2
Craugastoridae (1 species)											
<i>Craugastor augusti</i>				+							1
Eleutherodactylidae (3 species)											
<i>Eleutherodactylus cystignathoides</i>										+	1
<i>Eleutherodactylus gutttilatus</i>				+			+				2
<i>Eleutherodactylus longipes</i> *							+		+		2
Hylidae (4 species)											
<i>Acris blanchardi</i>										+	1
<i>Dryophytes arenicolor</i>				+							1
<i>Rheohyla miotympanum</i> *									+		1
<i>Smilisca baudinii</i>									+		1
Microhylidae (1 species)											
<i>Gastrophryne olivacea</i>	+	+	+	+	+	+	+		+	+	9
Ranidae (2 species)											
<i>Lithobates berlandieri</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Lithobates catesbeianus</i> ***	+			+			+				3
Scaphiopodidae (2 species)											
<i>Scaphiopus couchii</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Spea multiplicata</i>	+	+	+	+	+	+	+	+	+	+	10
Caudata (4 species)											
Ambystomatidae (1 species)											
<i>Ambystoma velasci</i> *								+			1
Plethodontidae (3 species)											
<i>Aquiloerycea galeanae</i> *									+		1
<i>Aquiloerycea scandens</i> *									+		1
<i>Chiropterotriton priscus</i> *									+		1
Squamata (106 species)											
Anguidae (5 species)											
<i>Barisia ciliaris</i> *									+		1
<i>Gerrhonotus infernalis</i>	+	+	+	+	+	+	+	+	+		9
<i>Gerrhonotus lugoi</i> **				+							1
<i>Gerrhonotus mccoysi</i> **				+							1
<i>Gerrhonotus parvus</i> *									+		1
Crotaphytidae (4 species)											
<i>Crotaphytus antiquus</i> **			+								1
<i>Crotaphytus collaris</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Crotaphytus reticulatus</i>										+	1
<i>Gambelia wislizenii</i>				+							1
Eublepharidae (2 species)											
<i>Coleonyx brevis</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Coleonyx reticulatus</i>				+							1
Gekkonidae (1 species)											

The herpetofauna of Coahuila, Mexico

Table 4 (continued). Distribution of the amphibians, squamates, and turtles of Coahuila, Mexico, by physiographic region. Abbreviations are as follows: BDM = Bolsón de Mapimi; LSV = Llanuras y Sierras Volcánicas; LDM = Laguna de Mayrán; SLC = Sierras y Llanuras Coahuilenses; SDB = Serranías del Burro; SLP = Sierra de la Paila; PSP = Pliegues Saltillo Parras; STR = Sierras Transversales; GSP = Gran Sierra Plegada; and LCN = Llanuras de Coahuila y Nuevo León. * = species endemic to Mexico; ** = species endemic to Coahuila; and *** = non-native species. See text for detailed descriptions of these regions.

Taxa	Physiographic Regions of Coahuila										Number of Regions Occupied
	BDM	LSV	LDM	SLC	SDB	SLP	PSP	STR	GSP	LCN	
Phrynosomatidae (29 species)											
<i>Cophosaurus texanus</i>	+	+	+	+	+	+	+	+	+		9
<i>Holbrookia approximans</i> *	+	+	+	+	+	+	+	+	+		9
<i>Holbrookia lacerata</i>										+	1
<i>Phrynosoma cornutum</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Phrynosoma modestum</i>	+	+	+	+	+	+	+	+	+		9
<i>Phrynosoma orbiculare</i> *								+	+		2
<i>Sceloporus cautus</i> *								+			1
<i>Sceloporus couchii</i> *				+		+	+	+			4
<i>Sceloporus cowlesi</i>	+	+	+	+	+	+	+	+	+		9
<i>Sceloporus cyanogenys</i>				+						+	2
<i>Sceloporus cyanostictus</i> *							+	+			2
<i>Sceloporus gadsdeni</i> **	+										1
<i>Sceloporus goldmani</i> *								+			1
<i>Sceloporus grammicus</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Sceloporus maculosus</i> *	+							+			2
<i>Sceloporus magister</i>		+		+		+					3
<i>Sceloporus marmoratus</i>										+	1
<i>Sceloporus merriami</i>				+		+		+		+	4
<i>Sceloporus minor</i> *									+		1
<i>Sceloporus olivaceus</i>				+	+						2
<i>Sceloporus ornatus</i> *							+	+			2
<i>Sceloporus parvus</i> *							+		+		2
<i>Sceloporus poinsettii</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Sceloporus samcolemanni</i> *								+			1
<i>Sceloporus spinosus</i> *								+			1
<i>Uma exsul</i> **	+										1
<i>Uma paraphygas</i> *	+										1
<i>Urosaurus ornatus</i>		+		+	+						3
<i>Uta stansburiana</i>	+	+	+	+		+	+	+	+		8
Scincidae (3 species)											
<i>Plestiodon dicei</i> *									+		1
<i>Plestiodon obsoletus</i>	+	+	+	+	+	+	+	+		+	9
<i>Plestiodon tetragrammus</i>				+	+					+	3
Sphenomorphidae (3 species)											
<i>Scincella kikaapoa</i> **				+							1
<i>Scincella lateralis</i>				+							1
<i>Scincella silvicola</i> *									+		1
Teiidae (4 species)											
<i>Aspidoscelis gularis</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Aspidoscelis inornata</i>	+	+	+	+	+	+	+	+	+		9
<i>Aspidoscelis marmorata</i>	+	+	+	+	+	+	+	+			8
<i>Aspidoscelis tessellata</i>		+		+							2
Xantusiidae (1 species)											
<i>Xantusia extorris</i> *								+			1
Colubridae (29 species)											
<i>Arizona elegans</i>	+	+	+	+	+	+	+			+	8
<i>Bogertophis subocularis</i>	+	+	+	+	+	+	+				7
<i>Coluber constrictor</i>				+							1

Table 4 (continued). Distribution of the amphibians, squamates, and turtles of Coahuila, Mexico, by physiographic region. Abbreviations are as follows: BDM = Bolsón de Mapimi; LSV = Llanuras y Sierras Volcánicas; LDM = Laguna de Mayrán; SLC = Sierras y Llanuras Coahuilenses; SDB = Serranías del Burro; SLP = Sierra de la Paila; PSP = Pliegues Saltillo Parras; STR = Sierras Transversales; GSP = Gran Sierra Plegada; and LCN = Llanuras de Coahuila y Nuevo León. * = species endemic to Mexico; ** = species endemic to Coahuila; and *** = non-native species. See text for detailed descriptions of these regions.

Taxa	Physiographic Regions of Coahuila										Number of Regions Occupied
	BDM	LSV	LDM	SLC	SDB	SLP	PSP	STR	GSP	LCN	
<i>Gyalopion canum</i>				+						+	2
<i>Lampropeltis alterna</i>				+			+				2
<i>Lampropeltis annulata</i>				+						+	2
<i>Lampropeltis gentilis</i>										+	1
<i>Lampropeltis leonis</i> *									+		1
<i>Lampropeltis splendida</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Masticophis flagellum</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Masticophis schotti</i>										+	1
<i>Masticophis taeniatus</i>	+	+	+	+							4
<i>Opheodrys aestivus</i>				+							1
<i>Pantherophis bairdi</i>				+							1
<i>Pantherophis emoryi</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Pituophis catenifer</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Pituophis deppei</i> *								+	+		2
<i>Rhinocheilus lecontei</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Salvadora deserticola</i>										+	1
<i>Salvadora grahamiae</i>				+							1
<i>Sonora episcopa</i>	+	+	+	+	+	+	+	+			8
<i>Tantilla atriceps</i>				+							1
<i>Tantilla cucullata</i>										+	1
<i>Tantilla gracilis</i>										+	1
<i>Tantilla hobartsmithi</i>				+							1
<i>Tantilla nigriceps</i>										+	1
<i>Tantilla wilcoxi</i>							+				1
<i>Trimorphodon vilkinsonii</i>										+	1
Dipsadidae (4 species)											
<i>Diadophis punctatus</i>				+	+		+				3
<i>Heterodon kennerlyi</i>	+	+	+	+	+	+	+	+		+	9
<i>Hypsiglena jani</i>	+	+	+	+	+	+	+	+	+		9
<i>Leptodeira septentrionalis</i>				+							1
Elapidae (1 species)											
<i>Micrurus tener</i>				+					+	+	3
Leptotyphlopidae (3 species)											
<i>Rena dissecta</i>				+							1
<i>Rena dulcis</i>				+						+	2
<i>Rena segregata</i>		+		+	+						3
Natricidae (7 species)											
<i>Nerodia erythrogaster</i>				+						+	2
<i>Nerodia rhombifer</i>										+	1
<i>Storeria hidalgoensis</i> *									+		1
<i>Thamnophis cyrtopsis</i>				+							1
<i>Thamnophis exsul</i> *									+		1
<i>Thamnophis marci</i>	+	+	+	+	+	+	+	+	+	+	10
<i>Thamnophis proximus</i>				+						+	2
Viperidae (10 species)											
<i>Agkistrodon laticinctus</i>				+	+						2
<i>Crotalus atrox</i>	+	+	+	+	+	+	+	+	+		9
<i>Crotalus lepidus</i>				+			+		+		3
<i>Crotalus molossus</i>								+	+		2

Table 4 (continued). Distribution of the amphibians, squamates, and turtles of Coahuila, Mexico, by physiographic region. Abbreviations are as follows: BDM = Bolsón de Mapimi; LSV = Llanuras y Sierras Volcánicas; LDM = Laguna de Mayrán; SLC = Sierras y Llanuras Coahuilenses; SDB = Serranías del Burro; SLP = Sierra de la Paila; PSP = Pliegues Saltillo Parras; STR = Sierras Transversales; GSP = Gran Sierra Plegada; and LCN = Llanuras de Coahuila y Nuevo León. * = species endemic to Mexico; ** = species endemic to Coahuila; and *** = non-native species. See text for detailed descriptions of these regions.

Taxa	Physiographic Regions of Coahuila										Number of Regions Occupied
	BDM	LSV	LDM	SLC	SDB	SLP	PSP	STR	GSP	LCN	
<i>Crotalus ornatus</i>				+							1
<i>Crotalus pricei</i>									+		1
<i>Crotalus scutulatus</i>	+	+	+	+		+	+	+	+		8
<i>Crotalus viridis</i>				+							1
<i>Sistrurus tergeminus</i>				+							1
Testudines (13 species)											
Emydidae (6 species)											
<i>Pseudemys gorzugi</i>				+	+					+	3
<i>Terrapene coahuila</i> **				+							1
<i>Terrapene ornata</i>		+									1
<i>Trachemys gaigeae</i>		+		+							2
<i>Trachemys scripta</i> ***				+						+	2
<i>Trachemys taylori</i> **				+							1
Kinosternidae (3 species)											
<i>Kinosternon durangoense</i> *	+										1
<i>Kinosternon flavescens</i>				+						+	2
<i>Kinosternon hirtipes</i>								+			1
Testudinidae (2 species)											
<i>Gopherus berlandieri</i>				+	+		+			+	4
<i>Gopherus flavomarginatus</i> *	+										1
Trionychidae (2 species)											
<i>Apalone atra</i> **				+						+	2
<i>Apalone spinifera</i>				+							1

borders the US state of Texas, it is not surprising that all 18 of these species, including the introduced species *Hemidactylus turcicus*, also are distributed in the USA.

Of the 143 species comprising the Coahuilan herpetofauna, 91 (63.6%) are found in only one or two physiographic regions, which is of great conservation significance (see below). The mean regional occupancy is 3.5.

Single-region species: Limited distribution increases conservation concern. The number of species found in a single region varies from one (in the Laguna de Mayrán, Llanuras y Sierras Volcánicas, and Pliegues Saltillo-Parras) to 23 (in the Sierras y Llanuras Coahuilenses). No single-region species are found in the Serranías del Burro region. On the following lists, * = endemic to Mexico, but found in more than one state; and ** = endemic to Coahuila.

Of the 23 single-region species in the Sierras y Llanuras Coahuilenses listed here by taxonomic order, 17 are Mexican non-endemics and the other six are endemic only within the boundaries of Coahuila.

- Craugastor augusti*
- Dryophytes arenicolor*
- Gerrhonotus lugoi***

- Gerrhonotus mccoysi***
- Gambelia wislizenii*
- Coleonyx reticulatus*
- Scincella kikaapoa***
- Scincella lateralis*
- Coluber constrictor*
- Opheodrys aestivus*
- Pantherophis bairdi*
- Salvadora grahamiae*
- Tantilla atriceps*
- Tantilla hobartsmithi*
- Leptodeira septentrionalis*
- Rena dissecta*
- Thamnophis cyrtopsis*
- Crotalus ornatus*
- Crotalus viridis*
- Sistrurus tergeminus*
- Terrapene coahuila***
- Trachemys taylori***
- Apalone atra***

Of the 15 single-region species in the Gran Sierra Plegada listed here, 13 are country endemics and the other two are Mexican non-endemics.

- Rheohyla miotympanum**

Table 5. Summary of distribution occurrence of herpetofaunal families in Coahuila, Mexico, by physiographic province. See Table 4 for explanation of abbreviations.

Families	Number of Species	Distributional Occurrence									
		BDM	LSV	LDM	SLC	SDB	SLP	PSP	STR	GSP	LCN
Bufonidae	7	4	4	3	6	5	4	4	2	2	5
Craugastoridae	1	—	—	—	1	—	—	—	—	—	—
Eleutherodactylidae	3	—	—	—	1	—	—	2	—	1	1
Hylidae	4	—	—	—	1	—	—	—	—	2	1
Microhylidae	1	1	1	1	1	1	1	1	—	1	1
Ranidae	2	2	1	1	2	1	1	2	1	1	1
Scaphiropodidae	2	2	2	2	2	2	2	2	2	2	2
Subtotals	20	9	8	7	14	9	8	11	5	9	11
Ambystomatidae	1	—	—	—	—	—	—	—	1	—	—
Plethodontidae	3	—	—	—	—	—	—	—	—	3	—
Subtotals	4	—	—	—	—	—	—	—	1	3	—
Totals	24	9	8	7	14	9	8	11	6	12	11
Anguidae	5	1	1	1	3	1	1	1	1	3	—
Crotaphytidae	4	1	1	2	2	1	1	1	1	1	2
Eublepharidae	2	1	1	1	2	1	1	1	1	1	1
Gekkonidae	1	1	1	1	1	1	1	1	1	1	1
Phrynosomatidae	29	12	10	8	14	9	11	12	18	11	7
Scincidae	3	1	1	1	2	2	1	1	1	1	2
Sphenomorphidae	3	—	—	—	2	—	—	—	—	1	—
Teiidae	4	3	4	3	4	3	3	3	3	2	1
Xantusiidae	1	—	—	—	—	—	—	—	1	—	—
Subtotals	52	20	19	17	30	18	19	20	27	21	14
Colubridae	29	9	9	9	19	9	8	10	7	7	16
Dipsadidae	4	2	2	2	4	3	2	3	2	1	1
Elapidae	1	—	—	—	1	—	—	—	—	1	1
Leptotyphlopidae	3	—	1	—	3	1	—	—	—	—	1
Natricidae	7	1	1	1	4	1	1	1	1	3	4
Viperidae	10	2	2	2	7	2	2	3	3	6	—
Subtotals	54	14	15	14	38	16	13	17	13	18	23
Emydidae	6	—	2	—	5	1	—	—	—	—	2
Kinosternidae	3	1	—	—	1	—	—	—	1	—	1
Testudinidae	2	1	—	—	1	1	—	1	—	—	1
Trionychidae	2	—	—	—	2	—	—	—	—	—	1
Subtotals	13	2	2	—	9	2	—	1	1	—	5
Totals	119	36	36	31	77	36	32	38	41	39	42
Sum Totals	143	45	44	38	91	45	40	49	47	51	53

Smilisca baudinii
*Aquiloerycea galeanae**
*Aquiloerycea scandens**
*Chiropterotriton priscus**
*Barisia imbricata**
*Gerrhonotus parvus**
*Sceloporus minor**
*Plestiodon dicei**
*Scincella silvicola**
*Lampropeltis leonis**
*Storeria hidalgoensis**
*Thamnophis exsul**
*Crotalus morulus**
Crotalus pricei

All of the 12 single-region species in the Llanuras de Coahuila y Nuevo León listed here are non-endemic to Mexico:

Eleutherodactylus cystignathoides
Acris blanchardi
Crotaphytus reticulatus
Holbrookia lacerata
Sceloporus marmoratus
Masticophis schotti
Salvadora deserticola
Tantilla cucullata
Tantilla gracilis
Tantilla nigriceps
Trimorphodon vilkinsonii
Nerodia rhombifer

Six of the seven single-region species in the Sierras Transversales listed here are country endemics and the other is a non-endemic:

*Ambystoma velasci**
*Sceloporus cautus**
*Sceloporus goldmani**
*Sceloporus samcolemani**
*Sceloporus spinosus**
*Xantusia extorris**
Kinosternon hirtipes

Three of the five single-region species in the Bolsón de Mapimí are country endemics, and the other two are state endemics:

*Sceloporus gadsdeni***
*Uma exsul***
*Uma paraphygas**
*Kinosternon durangoense**
*Gopherus flavomarginatus**

The single-region species in the Laguna de Mayrán is a state endemic lizard:

Crotaphytus antiquus

The one single-region species in the Llanuras y Sierras Volcánicas is a non-endemic turtle:

Terrapene ornata

The one single-region species in the Pliegues Saltillo-Parras is a non-endemic snake:

Tantilla wilcoxi

Examination of the above-listed species indicates that of the 65 single-region species in Coahuila, 22 are country endemics and nine are state endemics. The remaining 34 are non-endemic species that are also distributed in the USA.

Coefficient of Biogeographic Resemblance. A Coefficient of Biogeographic Resemblance (CBR) matrix was created for examining herpetofaunal relationships among the 10 physiographic regions of Coahuila (Table 6) and these data were used to produce a UPGMA dendrogram (Fig. 12). As mentioned above, the numbers of species within the 10 physiographic regions of Coahuila range from a high of 91 species within the Sierras y Llanuras Coahuilenses (SLC) to a low of 38 within Laguna de Mayrán (LDM). The mean species richness number for all 10 regions is 50.3. The numbers of species shared between regions range from 20 to 45. The lowest value of 20 is found between only one pair of regions, the Llanuras de Coahuila y Nuevo León (LCN) and Gran Sierra Plegada (GSP). The highest number is also shared between only one pair of regions, the SLC and Serranías del Burro (SDB). The mean number of shared species among the 45 regional pairings is 32.9. The lowest number of 20 shared species between LCN and GSP makes biogeographic sense because these two regions are situated at opposite ends of the state, are not connected geographically, and are environmentally quite different: LCN contains subhumid lowland plains and hills versus GSP with semihumid to subhumid highland mountainous areas carved by deep valleys. Also, GSP has a much smaller area in Coahuila than does LCN. On the other hand, the two regions with the highest number of 45 shared species are SLC and SDB. These two regions share part of their borders and both contain similar ecological regimes. Unlike the situation in Tamaulipas (Terán-Juárez et al. 2016), the higher numbers of species in the regional pairings in Coahuila, with the exception of SLC (91 species), do not necessarily equate to higher numbers of shared species, which is more similar to the patterns shown in adjacent Nuevo León (Navárez-de los Reyes et al. 2016). This discrepancy is most likely due to the larger number of included physiographic regions, and the lower number of shared species from more distant regions. Reflecting this trend, the following

Table 6. Pair-wise comparison matrix of Coefficient of Biogeographic Resemblance (CBR) data of herpetofaunal relationships for the 10 physiographic regions in Coahuila, Mexico. Underlined values = number of species in each region; upper triangular matrix values = species in common between two regions; and lower triangular matrix values = CBR values. The formula for this algorithm is $CBR = 2C/N_1 + N_2$ (Duellman 1990), where C is the number of species in common to both regions, N_1 is the number of species in the first region, and N_2 is the number of species in the second region. See Table 4 for explanation of abbreviations and Fig. 12 for the UPGMA dendrogram produced from the CBR data.

	BDM	LSV	LDM	SLC	SDB	SLP	PSP	STR	GSP	LCN
BDM	<u>45</u>	38	37	38	34	36	37	33	29	22
LSV	0.85	<u>44</u>	37	42	36	37	36	32	29	22
LDM	0.89	0.90	<u>38</u>	37	34	36	36	32	29	22
SLC	0.56	0.62	0.57	<u>91</u>	45	40	44	34	31	40
SDB	0.76	0.81	0.82	0.66	<u>45</u>	35	37	30	27	28
SLP	0.85	0.88	0.92	0.61	0.74	<u>40</u>	38	34	29	24
PSP	0.79	0.80	0.83	0.63	0.79	0.85	<u>49</u>	35	32	24
STR	0.72	0.70	0.75	0.49	0.65	0.78	0.73	<u>47</u>	31	21
GSP	0.60	0.61	0.65	0.44	0.56	0.64	0.64	0.63	<u>51</u>	20
LCN	0.45	0.45	0.48	0.56	0.57	0.52	0.47	0.42	0.38	<u>53</u>

pairwise comparisons of regions are aligned in order of highest to lowest species richness (underlined values) and their corresponding numbers of shared species (in parentheses) with all other regions; see text and map for discussions on characteristics and sizes of the regions:

SLC 91: SDB (45), SLP (40), PSP (44), STR (34), GSP (31), LCN (40), LDM (32), LSV (42), BDM (38).

LCN 53: GSP (20), STR (21), PSP (24), SLP (24), SDB (28), SLC (40), LDM (22), LSV (23), BDM (22).

GSP 51: LCN (20), STR (31), PSP (32), SLP (29), SDB (27), SLC (31), LDM (29), LSV (29), BDM (29).

PSP 49: STR (35), GSP (32), LCN (24), SLP (38), SDB (37), SLC (44), LDM (36), LSV (36), BDM (37).

STR 47: GSP (31), LCN (21), PSP (35), SLP (34), SDB (30), SLC (34), LDM (32), LSV (32), BDM (33).

SDB 45: SLP (35), PSP (27), STR (30), GSP (27), LCN (28), SLC (45), LDM (34), LSV (36), BDM (34).

BDM 45: LSV (38), LDM (37), SLC (38), SDB (34), SLP (36), PSP (37), STR (33), GSP (29), LCN (22).

LSV 44: BDM (38), LDM (37), SLC (42), SDB (36), SLP (37), PSP (36), STR (32), GSP (29), LCN (22).

SLP 40: BDM (36), LSV (37), LDM (36), SLC (40), SDB (35), PSP (38), STR (34), GSP (29), LCN (24).

LDM 38: BDM (37), SLP (36), PSP (36), STR (32), GSP (29), LCN (22), SLC (37), SDB (34), LSV (34).

SLC, with its 91 species, is the largest physiographic region in Coahuila that shares borders to variable extents with five of the nine other regions in the state (LCN, SDB, PSP, LSV, SLP), including the 2nd and 4th most speciose regions (LCN, PSP). The 91 species in SLC reveals a large discrepancy between it and all nine other regions in the state. Ninety-one species is 38 more than found in LCN, the second most species-rich region with 53 species, whereas the total difference for all nine of the

other regions is only 15 species between the 53 species in LCN and the 38 species in LDM. LCN is a lowland region next to the Rio Grande with few montane landscapes, but it contains several generalist herpetofaunal species that also exist in adjacent montane regions of Coahuila at lower elevations. PSP is mostly separated from LCN by two other regions to its north (LDM, SLP); however, it shares a geographic connection through a northwestern extension of PSP in Nuevo León (Nevárez-de los Reyes et al. 2016).

The following data show ranges and mean numbers of shared species for each of the 10 regions listed above that are arranged according to increasing mean numbers (bold in parentheses) with underlined values referring to species richness in each region:

- Llanuras de Coahuila y Nuevo León (LCN) (53): 20–40 (**24.7**)
- Gran Sierra Plegada - GSP (51): 20–32 (**28.5**)
- Sierras Transversales - STR (47): 21–35 (**31.3**)
- Laguna de Mayrán - LGM (38): 22–37 (**33.3**)
- Bolsón de Mapimí - BDM (45): 22–38 (**33.8**)
- Serranías del Burro - SDB (45): 27–45 (**34.0**)
- Llanuras y Sierras Volcánicas - LSV (44): 22–42 (**34.3**)
- Sierra de la Paila - SLP (40): 24–40 (**34.3**)
- Pliegues de Saltillo Parras - PSP (49): 24–38 (**35.4**)
- Sierras y Llanuras Coahuilenses - SLC (91): 31–45 (**39.0**)

With the exception of SLC and PSP (1st and 4th highest in species richness, and 1st and 2nd highest mean numbers of shared species, respectively), the mean number of pairwise species comparisons between all other regions indicate that higher species richness in a region does not necessarily translate into a higher mean number of shared species when all regions are totaled. Apparent extreme examples of this are: LCN, GSP, and STR, respectively, having the 1st, 2nd, and 3rd highest numbers of species and lowest mean numbers of shared species. It makes sense that LCN would share fewer species with other regions in Coahuila because of its ecological uniqueness



Fig. 9. *Sierras y Llanuras Coahuilenses*. Vegetation in the Valley of Cuatrociénegas, in the municipality of Cuatrociénegas de Carranza. Photo by Elí García-Padilla.



Fig. 10. *Sierra de la Paila*. Vegetation in the Sierra de la Paila, in the municipality of Ramos Arizpe. Photo by Bernardo Marino (<http://gransierraplegada.org>).



Fig. 11. *Sierras Transversales*. Paso de Carneros, Matorral rosetophilous vegetation, with Chocha (*Yucca carnerosana*) and Lechuguilla (*Agave lechuguilla*), at Paso de Carneros, in the municipality of Saltillo. Photo by Manuel Nevárez de los Reyes.

associated with lower elevations and general differences in vegetation formations and topography, as well as herpetofaunal affinities to the United States northward across the Rio Grande. GSP has the smallest area of all regions in the state, but is much more extensive when considering it also exists in Nuevo León and Tamaulipas (Nevárez-de los Reyes et al. 2016; Terán-Juárez et al. 2016). STR is a slender montane region with high species richness positioned primarily within Coahuila across its entire southern border.

UPGMA Dendrogram. Based on the data in Table 6, a UPGMA dendrogram (Fig. 12) was created to illustrate the herpetofaunal resemblance patterns in a hierarchical fashion among the 10 physiographic regions of Coahuila (Fig. 1). The patterns are different when compared to those shown in the two other northern Mexico states bordering Texas that were covered in previous MCS publications: Nuevo León (Nevárez-de los Reyes et al. 2016) and Tamaulipas (Terán-Juárez et al. 2016). The Coahuila dendrogram shows the similarity relationships in descending order from the most similar regions, SLP clustering with LDM at a value of 0.92, down to the lowest value, where LCN clusters with all the other regions at a value of 0.48. In other words, there are no

distinct subgroupings within the dendrogram, which indicates on a biogeographic scale that there are no distinct subgroups composed of distributional units that share more closely related herpetofaunas. On the other hand, neighboring Nuevo León with seven regions has two distinct biogeographic subgroups, a southern unit containing two regions and a more northern unit containing five regions that clusters with the southern unit at the 0.37 similarity level. Tamaulipas, also with seven recognized regions, has the most complex pattern of herpetological similarity of these three states. Two biogeographic subgroups are found in what can be considered the northern and eastern sections of the state. One of those subgroups contains three regions that make up the majority of the state's area, while the other subgroup is comprised of two small disjunct highland regions that cluster together at the 0.55 level; and both are nested within one of the other subgroup's regions. Those two subgroups cluster with each other at the 0.46 similarity level. One of the two remaining regions, which make up the extreme southwestern sector of Tamaulipas, clusters independently with the other two biogeographic subgroups at the 0.44 level of herpetofaunal similarity. The last region, which is the southwestern-most section of the state, is the most distinctive of the seven, thereby clustering with the others at the 0.23 level of herpetological similarity.

In summary, the UPMGA dendrogram for Coahuila shows that the lowland non-montane region (LCN) bordering the Rio Grande and Texas is the most distinctive region in Coahuila as far as herpetological similarity goes, based on numbers of shared species. It also shows a pattern of similarity among regions in close proximity to each other that also share ecological parameters either within the state or through areas of the same physiographic region outside Coahuila.

Distribution Status Categorizations

The discussion of the distribution status of Coahuilan herpetofauna members uses the system developed by Alvarado-Díaz et al. (2013), and employed in all the

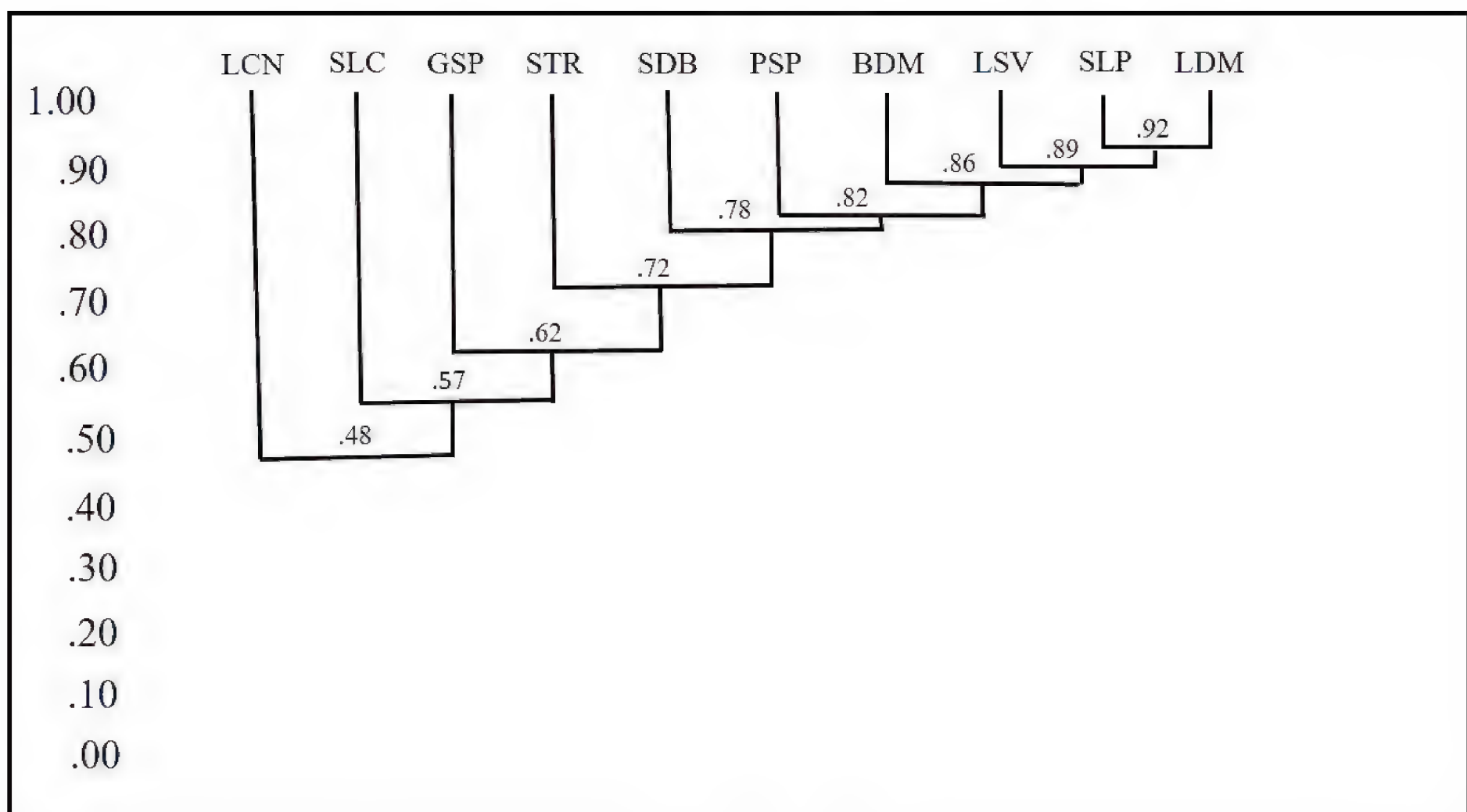


Fig. 12. A UPGMA generated dendrogram illustrating the similarity relationships of species richness among the herpetofauna in the 10 physiographic regions of Coahuila (based on the data in Table 6). We calculated the similarity values using Duellman's (1990) Coefficient of Biogeographic Resemblance (CBR).

other entries in the Mexican Conservation Series. The categories in the system are non-endemic, country endemic, state endemic, and non-native (Tables 7 and 8).

Given the 512-km-long border shared between Coahuila and Texas (<http://wikipedia.org>; accessed 11 August 2018), it is not surprising that the largest component of the herpetofauna falls into the non-endemic category. Of the 143 species comprising the Coahuilan herpetofauna, 100 species (69.9% of the total) belong to this category. Almost half (49) of the non-endemic species are snakes, and this number is only five fewer than the total number of snake species in the entire herpetofauna (Table 8). The 76 non-endemic squamate species are 71.7% of the total of 106 species for the state. In addition, a large portion of the state's anurans (17 of 20; 85.0%) are also non-endemic species. On the other hand, slightly more than half the turtle species (seven of 13; 53.8%) are non-endemic to Coahuila (Table 8).

The next largest component is the 31 (21.7%) country endemic species, most of which are squamates, including 18 lizards and five snakes (74.2%). The remainder are amphibians (six species; 19.4%) and turtles (two species; 6.5%). Almost half of the country endemics are phrynosomatid lizards (14 of 31; 45.2%).

Only nine of the species (6.3%) in Coahuila are state endemics. Six of these are lizards (*Gerrhonotus lugoi*, *G. mccoysi*, *Crotaphytus antiquus*, *Sceloporus gadsdeni*, *Uma exsul*, and *Scincella kikaapoa*) and three are turtles (*Terrapene coahuila*, *Trachemys taylori*, and *Apalone atra*).

The number of non-native species in Coahuila is only three, the ranid frog *Lithobates catesbeianus*, the

gekkonid lizard *Hemidactylus turcicus*, and the emydid turtle *Trachemys scripta*. These three species also were reported as introduced into Nuevo León (Nevárez-de los Reyes et al. 2016).

The number of endemic species in Coahuila (country and state endemics combined) is 40, which is 4.9% of the total number of endemic species for Mexico (811; Johnson unpub.). The number of non-endemic species is 100, which is 19.7% of the total of such species in the entirety of Mexico (508; Johnson unpub.).

Principal Environmental Threats

In this section we examine the 12 problems we think are the most significant in affecting the sustainability of the populations of Coahuila's amphibians and reptiles.

Urban development. As of 2015, the population of Coahuila was 2,954,915, making it the 16th most densely populated state in Mexico. The Municipality of Saltillo is the most populated of the 38 municipalities in the state, with a population of 807,537 people, followed by the municipalities of Torreón with 679,288, Monclova with 231,107, and Piedras Negras with 163,595 (see INEGI, <http://www.beta.inegi.org.mx/temas/estructura/>). Data from this same website indicate that 90% of the state population is located in urban areas, with the remainder in rural areas. The current annual percentage growth rate is 1.5%, which portends a doubling rate of about 47 years. Most of this growth is expected to occur within the most heavily-populated municipalities.

Table 7. Distributional and conservation status measures for members of the herpetofauna of Coahuila, Mexico. Distributional Status: SE = endemic to state of Coahuila; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN Categorization: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT Status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. * = species endemic to Mexico; ** = species endemic to Coahuila; *** = non-native species. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Taxa	Distributional Status	Environmental Vulnerability Category (Score)	IUCN Categorization	SEMARNAT Status
<i>Anaxyrus cognatus</i>	NE	L (9)	LC	NS
<i>Anaxyrus debilis</i>	NE	L (7)	LC	Pr
<i>Anaxyrus punctatus</i>	NE	L (5)	LC	NS
<i>Anaxyrus speciosus</i>	NE	M (12)	LC	NS
<i>Anaxyrus woodhousii</i>	NE	M (10)	LC	NS
<i>Incilius nebulifer</i>	NE	L (6)	LC	NS
<i>Rhinella horribilis</i>	NE	L (3)	LC	NS
<i>Craugastor augusti</i>	NE	L (8)	LC	NS
<i>Eleutherodactylus cystignathoides</i>	NE	M (12)	LC	NS
<i>Eleutherodactylus guttilatus</i>	NE	M (11)	LC	NS
<i>Eleutherodactylus longipes</i> *	CE	H (15)	VU	NS
<i>Acris blanchardi</i>	NE	M (12)	NE	NS
<i>Dryophytes arenicolor</i>	NE	L (7)	LC	NS
<i>Rheohyla miotympanum</i> *	CE	L (9)	NT	NS
<i>Smilisca baudinii</i>	NE	L (3)	LC	NS
<i>Gastrophryne olivacea</i>	NE	L (9)	LC	Pr
<i>Lithobates berlandieri</i>	NE	L (7)	LC	Pr
<i>Lithobates catesbeianus</i> ***	NN	—	—	—
<i>Scaphiopus couchii</i>	NE	L (3)	LC	NS
<i>Spea multiplicata</i>	NE	L (6)	LC	NS
<i>Ambystoma velasci</i> *	CE	M (10)	LC	Pr
<i>AquiloEURYCEA galeanae</i> *	CE	H (18)	NT	A
<i>AquiloEURYCEA scandens</i> *	CE	H (17)	VU	Pr
<i>Chiropterotriton priscus</i> *	CE	H (16)	NT	Pr
<i>Barisia ciliaris</i> *	CE	H (15)	NE	NS
<i>Gerrhonotus infernalis</i>	NE	M (13)	LC	NS
<i>Gerrhonotus lugoi</i> **	SE	H (16)	LC	NS
<i>Gerrhonotus mccoysi</i> **	SE	H (17)	NE	NS
<i>Gerrhonotus parvus</i> *	CE	H (17)	EN	Pr
<i>Crotaphytus antiquus</i> **	SE	H (16)	EN	NS
<i>Crotaphytus collaris</i>	NE	M (13)	LC	A
<i>Crotaphytus reticulatus</i>	NE	M (12)	VU	A
<i>Gambelia wislizenii</i>	NE	M (13)	LC	Pr
<i>Coleonyx brevis</i>	NE	H (14)	LC	Pr
<i>Coleonyx reticulatus</i>	NE	H (15)	LC	Pr
<i>Hemidactylus turcicus</i> ***	NN	—	—	—
<i>Cophosaurus texanus</i>	NE	H (14)	LC	A
<i>Holbrookia approximans</i> *	CE	H (14)	NE	NS
<i>Holbrookia lacerata</i>	NE	H (14)	NT	A
<i>Phrynosoma cornutum</i>	NE	M (11)	LC	NS
<i>Phrynosoma modestum</i>	NE	M (12)	LC	NS
<i>Phrynosoma orbiculare</i> *	CE	M (12)	LC	A
<i>Sceloporus cautus</i> *	CE	H (15)	LC	NS
<i>Sceloporus couchii</i> *	CE	H (15)	LC	NS
<i>Sceloporus cowlesi</i>	NE	M (13)	NE	NS
<i>Sceloporus cyanogenys</i>	NE	M (13)	NE	NS
<i>Sceloporus cyanostictus</i> *	CE	H (16)	EN	NS

Table 7 (continued). Distributional and conservation status measures for members of the herpetofauna of Coahuila, Mexico. Distributional Status: SE = endemic to state of Coahuila; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN Categorization: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT Status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. * = species endemic to Mexico; ** = species endemic to Coahuila; *** = non-native species. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Taxa	Distributional Status	Environmental Vulnerability Category (Score)	IUCN Categorization	SEMARNAT Status
<i>Sceloporus gadsdeni</i> **	SE	H (17)	NE	NS
<i>Sceloporus goldmani</i> *	CE	H (15)	EN	NS
<i>Sceloporus grammicus</i>	NE	L (9)	LC	Pr
<i>Sceloporus maculosus</i> *	CE	H (16)	VU	Pr
<i>Sceloporus magister</i>	NE	L (9)	LC	NS
<i>Sceloporus marmoratus</i>	NE	M (11)	NE	NS
<i>Sceloporus merriami</i>	NE	M (13)	LC	NS
<i>Sceloporus minor</i> *	CE	H (14)	LC	NS
<i>Sceloporus olivaceus</i>	NE	M (13)	LC	NS
<i>Sceloporus ornatus</i> *	CE	H (16)	NT	A
<i>Sceloporus parvus</i> *	CE	H (15)	LC	NS
<i>Sceloporus poinsettii</i>	NE	M (12)	LC	NS
<i>Sceloporus samcolemanni</i> *	CE	H (15)	LC	NS
<i>Sceloporus spinosus</i> *	CE	M (12)	LC	NS
<i>Uma exsul</i> **	SE	H (16)	EN	P
<i>Uma paraphygas</i> *	CE	H (17)	NT	P
<i>Urosaurus ornatus</i>	NE	M (10)	LC	NS
<i>Uta stansburiana</i>	NE	L (7)	LC	A
<i>Plestiodon dicei</i> *	CE	M (12)	NE	NS
<i>Plestiodon obsoletus</i>	NE	M (11)	LC	NS
<i>Plestiodon tetragrammus</i>	NE	M (12)	LC	NS
<i>Scincella kikaapoa</i> **	SE	H (17)	NE	NS
<i>Scincella lateralis</i>	NE	M (13)	LC	Pr
<i>Scincella silvicola</i> *	CE	M (12)	LC	A
<i>Aspidoscelis gularis</i>	NE	L (9)	LC	NS
<i>Aspidoscelis inornata</i>	NE	H (14)	LC	NS
<i>Aspidoscelis marmorata</i>	NE	H (14)	NE	NS
<i>Aspidoscelis tessellata</i>	NE	H (14)	LC	NS
<i>Xantusia extorris</i> *	CE	H (15)	LC	NS
<i>Arizona elegans</i>	NE	L (5)	LC	NS
<i>Bogertophis subocularis</i>	NE	H (14)	LC	NS
<i>Coluber constrictor</i>	NE	M (10)	LC	A
<i>Drymarchon melanurus</i>	NE	L (6)	LC	NS
<i>Gyalopion canum</i>	NE	L (9)	LC	NS
<i>Lampropeltis alterna</i>	NE	H (14)	LC	A
<i>Lampropeltis annulata</i>	NE	M (12)	NE	NS
<i>Lampropeltis gentilis</i>	NE	L (9)	NE	NS
<i>Lampropeltis leonis</i> *	CE	H (16)	NE	NS
<i>Lampropeltis splendida</i>	NE	M (12)	NE	NS
<i>Masticophis flagellum</i>	NE	L (8)	LC	A
<i>Masticophis schotti</i>	NE	M (13)	LC	NS
<i>Masticophis taeniatus</i>	NE	M (10)	LC	NS
<i>Opheodrys aestivus</i>	NE	M (13)	LC	NS
<i>Pantherophis bairdi</i>	NE	H (15)	LC	NS
<i>Pantherophis emoryi</i>	NE	M (13)	LC	NS
<i>Pituophis catenifer</i>	NE	L (9)	LC	NS
<i>Pituophis deppei</i> *	CE	H (14)	LC	A

The herpetofauna of Coahuila, Mexico

Table 7 (continued). Distributional and conservation status measures for members of the herpetofauna of Coahuila, Mexico. Distributional Status: SE = endemic to state of Coahuila; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN Categorization: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT Status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. * = species endemic to Mexico; ** = species endemic to Coahuila; *** = non-native species. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Taxa	Distributional Status	Environmental Vulnerability Category (Score)	IUCN Categorization	SEMARNAT Status
<i>Rhinocheilus lecontei</i>	NE	L (8)	LC	NS
<i>Salvadora deserticola</i>	NE	H (14)	NE	NS
<i>Salvadora grahamiae</i>	NE	M (10)	LC	NS
<i>Sonora episcopa</i>	NE	M (13)	NE	NS
<i>Tantilla atriceps</i>	NE	M (11)	LC	A
<i>Tantilla cucullata</i>	NE	M (12)	LC	NS
<i>Tantilla gracilis</i>	NE	M (13)	LC	A
<i>Tantilla hobartsmithi</i>	NE	M (11)	LC	NS
<i>Tantilla nigriceps</i>	NE	M (11)	LC	NS
<i>Tantilla wilcoxi</i>	NE	M (10)	LC	NS
<i>Trimorphodon wilkinsonii</i>	NE	H (15)	LC	A
<i>Diadophis punctatus</i>	NE	L (4)	LC	NS
<i>Heterodon kennerlyi</i>	NE	M (11)	NE	NS
<i>Hypsiglena jani</i>	NE	L (6)	NE	NS
<i>Leptodeira septentrionalis</i>	NE	L (8)	NE	NS
<i>Micrurus tener</i>	NE	M (11)	LC	NS
<i>Rena dissecta</i>	NE	M (11)	LC	NS
<i>Rena dulcis</i>	NE	M (13)	LC	NS
<i>Rena segregata</i>	NE	L (8)	NE	NS
<i>Nerodia erythrogaster</i>	NE	M (11)	LC	A
<i>Nerodia rhombifer</i>	NE	M (10)	LC	NS
<i>Storeria hidalgoensis</i> *	CE	M (13)	VU	NS
<i>Thamnophis cyrtopsis</i>	NE	L (7)	LC	NS
<i>Thamnophis exsul</i> *	CE	H (16)	LC	A
<i>Thamnophis marcianus</i>	NE	M (10)	LC	A
<i>Thamnophis proximus</i>	NE	L (7)	LC	A
<i>Agkistrodon laticinctus</i>	NE	H (15)	NE	NS
<i>Crotalus atrox</i>	NE	L (9)	LC	Pr
<i>Crotalus lepidus</i>	NE	M (12)	LC	Pr
<i>Crotalus molossus</i>	NE	L (8)	LC	Pr
<i>Crotalus morulus</i> *	CE	H (16)	NE	NS
<i>Crotalus ornatus</i>	NE	M (13)	NE	NS
<i>Crotalus pricei</i>	NE	H (14)	LC	Pr
<i>Crotalus scutulatus</i>	NE	M (11)	LC	Pr
<i>Crotalus viridis</i>	NE	M (12)	LC	Pr
<i>Sistrurus tergeminus</i>	NE	M (13)	LC	Pr
<i>Pseudemys gorzugi</i>	NE	H (16)	NT	A
<i>Terrapene coahuila</i> **	SE	H (19)	EN	A
<i>Terrapene ornata</i>	NE	H (15)	NT	Pr
<i>Trachemys gaigeae</i>	NE	H (18)	VU	NS
<i>Trachemys scripta</i> ***	NN	—	—	—
<i>Trachemys taylori</i> **	SE	H (19)	EN	NS
<i>Kinosternon durangoense</i> *	CE	H (16)	DD	NS
<i>Kinosternon flavescens</i>	NE	M (12)	LC	NS
<i>Kinosternon hirtipes</i>	NE	M (10)	LC	Pr
<i>Gopherus berlandieri</i>	NE	H (18)	LC	A
<i>Gopherus flavomarginatus</i> *	CE	H (19)	VU	P
<i>Apalone atra</i> **	SE	H (20)	NE	P
<i>Apalone spinifera</i>	NE	H (15)	LC	Pr



Fig. 13. Urban Development. Urban growth near Saltillo, reaching the limit of the Natural Protected Area “Sierra de Zapalinamé,” in the municipality of Saltillo. *Photo by Manuel Nevárez de los Reyes.*



Fig. 14. Industrial Pollution. Factories polluting the air in the vicinity of Monclova, in the municipality of Monclova. *Photo by Michael Price.*



Fig. 15. Deforestation for Agricultural Purposes. Monoculture of grapes near Parras de la Fuente, in the municipality of Parras. *Photo by Manuel Nevárez de los Reyes.*



Fig. 16. Deforestation for Agricultural Purposes. Monoculture of cotton at San Pedro de las Colonias, in the municipality of the same name, Provincia de Laguna de Mayran. *Photo by Manuel Nevárez de los Reyes.*

Urban pollution. One outcome of urban growth that is of environmental significance is the accumulation of garbage resulting from the inefficient use of resources by populations in urban areas. An extensive recent study acknowledged that “*garbage is the major environmental problem facing Mexico, involving the generation of more than 100 million tons of wastes per year that are not handled in an adequate manner*” (<http://www.estosdias.com.mx/blog/archivos/226>; accessed 18 September 2018). This site points out that the Federal District and its metropolitan area has the largest garbage dump in the world, located in Ciudad Nezahualcóyotl in the State of México. The useful life of this site has been extended despite the lack of additional space being available, which points out the difficulty of finding other sites to deposit the thousands of tons of garbage produced. With reference to Coahuila, in an article published in *Torreón* on 14 August 2018 by *Noticias—El Sol de la Laguna*, the Secretary of the Environment, Eglantina Canales Gutiérrez, reported that 3,000 tons of garbage are produced in the state of Coahuila on a daily basis, or almost one kilogram of trash produced per person

per day. Secretary Canales stated that the burial of such trash in landfills, which are available in 85% of Coahuila, represents the best solution to date, but that in the future efforts to recycle products should be implemented. She decried that often garbage does not end up in landfills, but rather is left out in the open. This environmental problem can be expected to grow commensurate with the rate of human population growth in Coahuila.

Several instances of the direct impact of accumulated garbage on members of the Mexican herpetofauna have been documented. Lazcano et al. (2006) reported the death of several Texas Horned Lizards (*Phrynosoma cornutum*), that were trapped inside a discarded tire in an illegal dump site in the neighboring state of Nuevo León. The lizards, presumably seeking shelter, died after they were unable to escape due to the intense daily temperatures at this locality that can rise to 45 °C in the shade. On another occasion in Nuevo León, Chávez-Cisneros et al. (2010) reported finding a Greater Earless Lizard (*Cophosaurus texanus*) that apparently died after ingesting a deflated balloon left in a pile of litter. The full environmental impact of discarded trash on the native

Table 8. Summary of the distributional status of herpetofaunal families in Coahuila, Mexico.

Families	Number of Species	Distributional Status			
		Non-endemic (NE)	Country Endemic (CE)	State Endemic (SE)	Non-native (NN)
Bufonidae	7	7	—	—	—
Craugastoridae	1	1	—	—	—
Eleutherodactylidae	3	2	1	—	—
Hylidae	4	3	1	—	—
Microhylidae	1	1	—	—	—
Ranidae	2	1	—	—	1
Scaphiopodidae	2	2	—	—	—
Subtotals	20	17	2	—	1
Ambystomatidae	1	—	1	—	—
Plethodontidae	3	—	3	—	—
Subtotals	4	—	4		
Totals	24	17	6	—	1
Anguidae	5	1	2	2	—
Crotaphytidae	4	3	—	1	—
Eublepharidae	2	2	—	—	—
Gekkonidae	1	—	—	—	1
Phrynosomatidae	29	14	13	2	—
Scincidae	3	2	1	—	—
Sphenomorphidae	3	1	1	1	—
Teiidae	4	4	—	—	—
Xantusiidae	1	—	1	—	—
Subtotals	52	27	18	6	1
Colubridae	29	27	2	—	—
Dipsadidae	4	4	—	—	—
Elapidae	1	1	—	—	—
Leptotyphlopidae	3	3	—	—	—
Natricidae	7	5	2	—	—
Viperidae	10	9	1	—	—
Subtotals	54	49	5	—	—
Emydidae	6	3	—	2	1
Kinosternidae	3	2	1	—	—
Testudinidae	2	1	1	—	—
Trionychidae	2	1	—	1	—
Subtotals	13	7	2	3	1
Totals	119	83	25	9	2
Sum Totals	143	100	31	9	3

herpetofauna of Coahuila, and elsewhere in Mexico, is still unknown, so it is imperative that actions be taken to diminish this overtly intentional behavior by an uncaring populace.

Industrial pollution. The municipalities of Acuña, Monclova, Piedras Negras, Ramos Arizpe, Saltillo, and Torreón have a Vehicle Verification Program (PVV) to address vehicular air pollution, which is supervised under municipal authority (Anon 2017).

Coahuila produces 16% of the electric power and 23% of the steel in Mexico. The industry dedicated to the extraction and commercialization of coal, in the coal region around Nava and Piedras Negras, has

been named as the one that most intensively pollutes the air (Journalistic note of May 4, 2016, <http://www.vanguardia.com.mx/articulo/empresas-carboneras-las-que-mas-contaminan-el-aire-en-la-carbonifera-y-norte-de-coahuila>).

Deforestation for agricultural and ranching purposes. One of the most notorious cases of massive deforestation carried out in the state of Coahuila happened in 2001. It occurred in the Valle del Hundido, adjacent to the Cuatro Ciénegas Valley, where hundreds of hectares were cleared for the establishment of alfalfa crops to feed dairy and beef cattle (<http://www.vanguardia.com.mx/columnas-elhundido-1728357.html>). Another case



No. 9. *Phrynosoma orbiculare* (Linnaeus, 1758). The Mountain Horned Lizard is a Mexican endemic species that occurs “from eastern Sonora and western Chihuahua southward through the mountains of Durango, Zacatecas, Aguascalientes, Jalisco, and Michoacán, and from the mountains of southern Nuevo León southward through San Luis Potosí, Querétaro, Hidalgo, Veracruz, and westward through Puebla, Tlaxcala, Mexico, the Distrito Federal, and Morelos” (Lemos-Espinal and Dixon 2013: 122). Bryson et al. (2011) noted, however, that this species is probably comprised of several distinct lineages, of which some appear to have small distributions and long independent evolutionary histories, and that some of these lineages merit additional consideration for protection. This individual was found near Monterreal, in the municipality of Arteaga. Wilson et al. (2013a) calculated its EVS as 12, placing it in the upper half of the medium vulnerability category. Its conservation status has been reported as Least Concern by IUCN, and as threatened (A) by SEMARNAT. *Photo by Elí García-Padilla.*



No. 10. *Sceloporus cautus* Smith, 1938. The Shy Spiny Lizard is an endemic Mexican lizard distributed from “the western slopes of the Sierra Madre Oriental in Tamaulipas, southeastern Coahuila, and central Nuevo León southward through much of San Luis Potosí and the northern half of Zacatecas” (Lemos-Espinal and Dixon 2013: 124). This individual was found at Cañon el Chorro, in the municipality of Arteaga. Wilson et al. (2013a) estimated its EVS as 15, placing it in the lower portion of the high vulnerability category. Its conservation status has been gauged as Least Concern by IUCN, but it is not listed by SEMARNAT. *Photo by Michael S. Price.*



No. 11. *Sceloporus olivaceus* Smith, 1934. The Texas Spiny Lizard is found from “northern central Texas southward through the Gulf of Mexico coastal plain to southern Tamaulipas, westward nearly to the Big Bend area of Texas and eastern Coahuila” (Lemos-Espinal et al. 2015: 239). This individual was encountered at Cañon el Chorro, in the municipality of Arteaga. Wilson et al. (2013a) calculated its EVS as 13, placing it at the upper limit of the medium vulnerability category. Its conservation status has been considered as Least Concern by IUCN; this species is not listed by SEMARNAT. *Photo by Michael S. Price.*



No. 12. *Sceloporus ornatus* Baird, 1859. The Ornate Spiny Lizard is a Mexican endemic species distributed in “southern and central Coahuila” (Lemos-Espinal and Smith 2007: 303). This individual was found at ca. 8 km SW from Ejido Mayrán, in the municipality of San Pedro. Wilson et al. (2013a) determined its EVS as 16, placing it in the middle portion of the high vulnerability category. Its conservation status has been calculated as Near Threatened by IUCN and as threatened (A) by SEMARNAT. *Photo by Marco Antonio Bazán-Tellez.*

involved members of the Mennonite community in Coahuila who were implicated in the clearing of 2,300 hectares of forest vegetation without authorization in the municipality of Sierra Mojada; however, they managed to escape legal sanction (<https://www.eluniversal.com.mx/estados/menonitas-de-coahuila-ganan-amparos-la-profepa-por-2-mil-hectareas-de-predios>).

Effect of roads. The state of Coahuila has an extensive network of roads and highways, with a total of seven federal highways. The total length of the road network in the state is 8,336 km (Servicio Geológico Mexicano 2017). During the period from 1980 to 2015, the number of motor vehicles traveling within Coahuila went from 149,242 to 741,515, an increase of 592,273 (or almost 400%!) in 35 years. The effect of roads on the herpetofauna of the state remains to be studied, but we assume that major highways might disrupt general dispersion patterns and seasonal migration of some local populations, and vehicles on municipal roads probably simply run over many animals in large numbers.

Mining and energy projects. The history of Coahuila is closely related to mining. It began as a leading commercial activity in the colonial era, with the founding of the city of Monclova and Minas de la Trinidad in 1577, and later continued with the discovery and exploitation of coal starting in 1828; copper in the Pánuco mine in 1870; zinc oxide, silver, and lead in Sierra Mojada in 1879; and silver, lead, and zinc in Reforma-Santa Teresa in 1890. Most recently, the discovery and exploitation of fluorite, celestite, sodium-magnesium salts, gypsum, barite, and dolomite has been undertaken. Coahuila has an extensive mining-metallurgical infrastructure, highlighted by the metal foundry in Torreón, the iron foundry in Monclova, coal plants in Nava, and several related processing plants in various locations. The state of Coahuila contributed 3.1% of the value of national mining production in 2015, occupying the first place in the production of iron, coal, celestite, magnesium sulfate, sodium sulfate, bismuth, and cadmium, second place in fluorite and silica, third in barite and dolomite, fifth in stone aggregates, and in smaller proportions plaster, sulfur, clays, gravel, sand, limestone, gold, and silver. In addition, a large number of unexplored geological areas have been reported in Coahuila (Servicio Geológico Mexicano 2017), for example, the Hercules mine in the northwestern portion of the state (<http://www.thediggings.com/mines/21966>; accessed 20 July 2019), and those places are expected to be sites for future development.

Natural gas production in the Burgos Basin. The Burgos Basin is a shale deposit located in the northeastern portion of Coahuila, directly south of the Rio Grande. It is considered a great prospect for natural gas production and covers a total area of 62,677 km². The infrastructure required to develop the production of natural gas is

scheduled to be built in a timely manner during successive 10-year periods, and involves extensive extraction, processing, and distribution facilities. It is expected that the previous and on-going infrastructure projects will have negative effects on the integrity of the habitat that is used by the members of the regional herpetofauna (<http://energiaadebate.com/gas-de-lutitas-en-la-cuenca-de-burgos/>).

Wind turbines. In 2016, a wind farm consisting of 95 wind turbines was being built near Ejido Hipólito in the Municipality of Ramos Arizpe, and another with 100 wind towers near Acuña, as well as a Solar Energy Park in the Municipality of Viesca, which will be the largest in Latin America and is planned to include more than two million solar panels (<http://coahuila.gob.mx/noticias/index/avanza-parque-eolico-de-hipolito-24-07-16>). The Ramos Arizpe project covers an area of 4,754 hectares. This requires, among other actions, the construction of more than 50 km of access roads, an aerial transmission line almost five km long, and an electrical substation (<http://proyectoecolico.coahuila.com>).

Elimination of herpetofauna due to cultural beliefs and practices. Traditional beliefs and practices that affect the herpetofauna of Coahuila are the same as those previously reported for Nuevo León (Nevárez-de los Reyes et al. 2016), likely due to their geographic proximity and similar cultural backgrounds. Examples include snakes being slaughtered due to fear and superstition, misconceptions that many non-venomous herpetofaunal species are venomous, and rattlesnakes being consumed either for food or the belief that their meat will prevent or cure cancer. It is obvious that education is the key for reducing the needless killing of these ecologically important denizens of Coahuila.

Use of pesticides. Agricultural activities in the state of Coahuila are most intense in the arid region known as Comarca Lagunera, where they are only possible due to heavy irrigation using water from the Río Nazas. Although there is little information regarding this problem, pesticides are commonly applied on Cantaloupe (*Cucumis melo*) fields, one of the most important fruits cultivated in the region (Vargas-González et al. 2016). That study found that in the agricultural cycle of 2010, 50 different active ingredients were used in the region; 26% of which were not authorized for use on cantaloupes by COFEPRIS, and 46% of which were considered as highly toxic to human health and the environment. For example, six of them (carbofuran, endosulfan, clorotalonil, mancozeb, imidacloprid, and metamidofos) are regarded as among the most toxic to humans and the environment.

Collecting and commercial trade. Currently, the herpetofauna of Coahuila faces problems with unlawful

collecting and commercial trade that are similar to those reported in Tamaulipas and Nuevo León (Terán-Juárez et al. 2016; Nevárez-de los Reyes et al. 2016). Unfortunately, these illegal activities have increased during the last few years through social media. For example, based on our personal observations, when photographs of rare species are posted, it is common for collectors to be immediately contacted by animal traffickers or pet owners ready to purchase their specimens, or asked for specific localities. Unfortunately, monitoring these activities remains difficult, as does determining their true impact on the local herpetofauna.

Fires involving natural habitats. Fires create a serious threat to natural habitats in Coahuila, especially those involving forested ecosystems. In 2017, 153 fires were documented in the state beginning on 2 January and ending on 23 December; thus, on average, a reported fire occurred somewhere in the state every 2.4 days. Of the 38 municipalities in Coahuila, fires were recorded in 22 (57.9%) of them. The municipalities most often involved were Arteaga (37 fires), Cuatro Ciénegas (12), Múzquiz (11), Ramos Arizpe (23), and Saltillo (24). Three of these municipalities (Arteaga, Ramos Arizpe, and Saltillo) are located in the extreme southeastern portion of the state where the state capital is located and where a majority of the fires have occurred (84 of 153 fires, or 54.9%). The number of ha involved in each of these 153 fires ranged from 0.02 to 3,132. The largest of these fires took place in the Municipality of San Buenaventura, at a locality called, interestingly enough, El Quemado (“burned by fire”). The total area burned in 2017 amounted to 10,289.6 ha, which is approximately 0.07% the total area of Coahuila (15,159,500 ha).

At the time of this writing, information is also available for the first 4.5 months of 2018 (10 January to 13 May) (CONAFOR, Comisión Nacional Forestal Aspectos de Incendios Forestales, <https://www.gob.mx/conafor>). During this period of time, 46 fires were reported, or one fire in Coahuila every 2.9 days. Fires were registered in 14 of the 38 municipalities in the state (36.8%). These fires occurred most often in the Municipalities of Múzquiz (15 fires), Cuatro Ciénegas (four), Arteaga (three), Nadadores (three), Ocampo (three), and Saltillo (three). Four of these six municipalities (Múzquiz, Cuatro Ciénegas, Arteaga, and Saltillo) represent areas with high fire occurrences during 2017 (see above). The number of hectares burned in each fire during this 2018 period varied from 0.5 to 1,500. The largest fire took place in Eutimias, in the Municipality of Ocampo, due to lightning strikes. In general, the principal causes of these fires, when known, were human negligence and lightning. During this period, the total area burned was 4,198.3 ha (about 0.03% of the state, or 31.3 ha/ day). This figure compares to 28.2 ha/day in 2017. It appears likely that the total number of hectares burned in Coahuila in 2018 should compare to those burned in 2017 (see above). The

threat posed by fires will almost assuredly increase into the future, given the human population growth in the state of 7.5% from 2010 to 2015, which is higher than the rate in Mexico as a whole (6.8%; <http://wikipedia.org>; accessed 5 July 2018).

Lazcano et al. (2006) reported finding a Wiegmann’s Alligator Lizard (*Gerrhonotus liocephalus*) killed by a forest fire in the neighboring state of Nuevo León and advised that only additional investigations would be able to determine the demographic consequences of this sort of mortality. Banda-Leal et al. (2018) documented for the first time the distribution of *Gerrhonotus parvus* in Coahuila, and mentioned finding some specimens of this species in the Sierra Zapalinamé that had died from forest fires.

Conservation Status

The discussion of the conservation status of members of the Coahuilan herpetofauna follows the same three systems of conservation assessment as used in the other entries in the Mexican Conservation Series. These systems are those found in SEMARNAT (2010), the IUCN Red List (<http://iucnredlist.org>), and the EVS (Wilson et al. 2013a,b). The assessments from these three systems were updated as needed.

The SEMARNAT System. SEMARNAT (Secretaría del Medio Ambiente y Recursos Naturales) is the environmental ministry of Mexico, and it is “*charged with the mission of protecting, restoring, and conserving the ecosystems, natural resources, assets and environmental services of Mexico with the goal of fostering sustainable development*” (<http://www.semarnat.gob.mx/conocenos/quienessomos>; accessed 7 January 2019). In 2010, this agency published the Norma Oficial Mexicana (Official Mexican Standard)-059, which deals with the protection of the native members of the Mexican flora and fauna and establishes categories of risk. This system is commonly used by Mexican herpetologists to discuss various segments of the country’s herpetofauna. Its utility for work on the herpetofauna of Coahuila has also been assessed. The SEMARNAT system comprises three categories, including Endangered (P), Threatened (A), and Special Protection (Pr). For a Mexican species not evaluated using one of these three categories, it is designated here as having no status (NS). The SEMARNAT evaluations are shown in Table 7 and summarized in Table 9.

Unfortunately, the SEMARNAT assessments are not very useful here, as most species in Coahuila remain unevaluated (90 of 140 native species; 64.3%). Thus, evaluations of conservation status are available for only 50 species (35.7%). Of these 50 species, 23 (46.0% of the total) are judged as species of Special Protection (Pr): *Anaxyrus debilis*, *Gastrophryne olivacea*, *Lithobates berlandieri*, *Ambystoma velasci**, *Aquiloerycea scandens**, *Chiropterotriton priscus**, *Gerrhonotus*



Fig. 17. *Deforestation for Ranching Purposes.* Goats in the vicinity of Estación Marte, in the municipality of General Cepeda. *Photo by Manuel Nevárez de los Reyes.*



Fig. 18. *Deforestation for Ranching Purposes.* Cattle in the municipality of Francisco I. Madero. *Photo by Manuel Nevárez de los Reyes.*



Fig. 19. *Impact of Roads.* *Masticophis flagellum* dead on the road near El Mimbres, in the municipality of Parras. *Photo by Manuel Nevárez de los Reyes.*



Fig. 20. *Mining Projects.* Mineral charcoal mining exploitation, in the municipality of Nava. *Photo by Manuel Nevárez de los Reyes.*



Fig. 21. *Mining Projects.* Mining of materials for construction near Paso de Carneros, in the municipality of Saltillo. *Photo by Manuel Nevárez de los Reyes.*



Fig. 22. *Energy Projects.* Wind generation of electricity near Hipolito, in the municipality of Ramos Arizpe. *Photo by Manuel Nevárez de los Reyes.*

parvus, *Gambelia wislizenii*, *Coleonyx brevis*, *Coleonyx reticulatus*, *Sceloporus grammicus*, *S. maculosus**, *Scincella lateralis*, *Crotalus atrox*, *C. lepidus*, *C. molossus*, *C. pricei*, *C. scutulatus*, *C. viridis*, *Sistrurus tergeminus*, *Terrapene ornata*, *Kinosternon hirtipes*, and *Apalone spinifera*. These 23 species include four that are endemic to Mexico (indicated by asterisks); and all of the 19 non-endemic species are shared with the United States. The

remaining 27 species are assessed as either Endangered (P) or Threatened (A). The Endangered species amount to four: *Uma exsul*, *U. paraphygae*, *Gopherus flavomarginatus*, and *Apalone atra* (Table 7); three are endemic to Coahuila and one is endemic to Mexico. Twenty-three species are considered as Threatened: *Aquiloeurycea galeanae*, *Crotaphytus collaris*, *C. reticulatus*, *Cophosaurus texanus*, *Holbrookia lacerata*,

Table 9. SEMARNAT categorizations for herpetofaunal species in Coahuila, Mexico, arranged by families. Non-native species are not included.

Families	Number of species	SEMARNAT Categorizations			
		Endangered (P)	Threatened (A)	Special protection (Pr)	No status (NS)
Bufonidae	7	—	—	1	6
Craugastoridae	1	—	—	—	1
Eleutherodactylidae	3	—	—	—	3
Hylidae	4	—	—	—	4
Microhylidae	1	—	—	1	—
Ranidae	1	—	—	1	—
Scaphiopodidae	2	—	—	—	2
Subtotals	19	—	—	3	16
Ambystomatidae	1	—	—	1	—
Plethodontidae	3	—	1	2	—
Subtotals	4	—	1	3	—
Totals	23	—	1	6	16
Anguidae	5	—	—	1	4
Crotaphytidae	4	—	2	1	1
Eublepharidae	2	—	—	2	—
Phrynosomatidae	29	2	5	2	20
Scincidae	3	—	—	—	3
Sphenomorphidae	3	—	1	1	1
Teiidae	4	—	—	—	4
Xantusiidae	1	—	—	—	1
Subtotals	51	2	8	7	34
Colubridae	29	—	7	—	22
Dipsadidae	4	—	—	—	4
Elapidae	1	—	—	—	1
Leptotyphlopidae	3	—	—	—	3
Natricidae	7	—	4	—	3
Viperidae	10	—	—	7	3
Subtotals	54	—	11	7	36
Emydidae	5	—	2	1	2
Kinosternidae	3	—	—	1	2
Testudinidae	2	1	1	—	—
Trionychidae	2	1	—	1	—
Subtotals	12	2	3	3	4
Totals	117	4	22	17	74
Sum Totals	140	4	23	23	90

Phrynosoma orbiculare, *Sceloporus ornatus*, *Uta stansburiana*, *Scincella silvicola*, *Coluber constrictor*, *Lampropeltis alterna*, *Masticophis flagellum*, *Pituophis deppei*, *Tantilla atriceps*, *T. gracilis*, *Trimorphodon wilkinsonii*, *Nerodia erythrogaster*, *Thamnophis exsul*, *T. marcianus*, *T. proximus*, *Pseudemys gorzugi*, *Terrapene coahuila*, and *Gopherus berlandieri*. This group of 23 species includes a curious mixture of seven country and state endemic species and 16 relatively broadly ranging non-endemic species, some with extensive ranges in the United States.

Until such time as SEMARNAT provides conservation evaluations for all Mexican herpetofaunal species, this system will continue to have only limited utility for conservation purposes.

The IUCN System. The system of conservation categorization developed by the International Union for the Conservation of Nature (IUCN) is applied globally, theoretically to all organisms, and consists of a set of nine categories, divided into four general categories, including: Extinct (Extinct and Extinct in the Wild), Threatened (Critically Endangered, Endangered, and Vulnerable), Lower Risk (Near Threatened and Least Concern), and other categories (Data Deficient and Not Evaluated). Those evaluations applying to the Coahuilan herpetofauna are shown in Table 7 and summarized in Table 10.

Only 14 of 140 native species (10.0%) are judged to be Threatened. No species are considered to be Critically Endangered; seven are assessed as Endangered; and seven

Table 10. IUCN Red List categorizations for herpetofaunal families in Coahuila, Mexico. Non-native species are excluded. The shaded columns to the left are the “threat categories,” and those to the right are the categories for which either too little information on conservation status exists to allow the taxa to be placed in any IUCN category, or they have not been evaluated.

Families	Number of Species	IUCN Red List categorizations						
		Critically Endangered	Endangered	Vulnerable	Near Threatened	Least Concern	Data Deficient	Not Evaluated
Bufonidae	7	—	—	—	—	7	—	—
Craugastoridae	1	—	—	—	—	1	—	—
Eleutherodactylidae	3	—	—	1	—	2	—	—
Hylidae	4	—	—	—	1	2	—	1
Microhylidae	1	—	—	—	—	1	—	—
Ranidae	1	—	—	—	—	1	—	—
Scaphiopodidae	2	—	—	—	—	2	—	—
Subtotals	19	—	—	1	1	16	—	1
Ambystomatidae	1	—	—	—	—	1	—	—
Plethodontidae	3	—	—	1	2	—	—	—
Subtotals	4	—	—	1	2	1	—	—
Totals	23	—	—	2	3	17	—	1
Anguidae	5	—	1	—	—	2	—	2
Crotaphytidae	4	—	1	1	—	2	—	—
Eublepharidae	2	—	—	—	—	2	—	—
Phrynosomatidae	29	—	3	1	3	17	—	5
Scincidae	3	—	—	—	—	2	—	1
Sphenomorphidae	3	—	—	—	—	2	—	1
Teiidae	4	—	—	—	—	3	—	1
Xantusiidae	1	—	—	—	—	1	—	—
Subtotals	51	—	5	2	3	31	—	10
Colubridae	29	—	—	—	—	23	—	6
Dipsadidae	4	—	—	—	—	1	—	3
Elapidae	1	—	—	—	—	1	—	—
Leptotyphlopidae	3	—	—	—	—	2	—	1
Natricidae	7	—	—	1	—	6	—	—
Viperidae	10	—	—	—	—	8	—	2
Subtotals	54	—	—	1	—	42	—	11
Emydidae	5	—	2	1	2	—	—	—
Kinosternidae	3	—	—	—	—	2	1	—
Testudinidae	2	—	—	1	—	1	—	—
Trionychidae	2	—	—	—	—	1	—	1
Subtotals	12	—	2	2	2	4	1	1
Totals	117	—	7	5	5	77	1	23
Sum Totals	140	—	7	7	8	94	1	23
Category Totals	140	14			102		24	

others as Vulnerable. The seven Endangered species are: *Gerrhonotus parvus**, *Crotaphytus antiquus***, *Sceloporus cyanostictus**, *S. goldmani**, *Uma exsul***, *Terrapene coahuila***, and *Trachemys taylori***. Three of these species are country endemics and four are state endemics. The seven Vulnerable species (VU) are: *Eleutherodactylus longipes**, *Aquiloerycea scandens**, *Crotaphytus reticulatus*, *Sceloporus maculosus**, *Storeria hidalgoensis**, *Trachemys gaigeae*, and *Gopherus flavomarginatus**. Five of the seven VU species are country endemics; the remainder are non-endemics. The Lower Risk species amount to 102 species, constituting 72.9% of the native species in the state (Table 10). Of these 102 species, eight are Near Threatened and 94

are Least Concern. As has been demonstrated clearly in other studies in the Mexican Conservation Series, the allocation of such a large number of species to the Least Concern category seems unjustified and the conservation status of these 94 “Least Concern” species is examined in greater detail in the following section.

Only a single Coahuilan species is considered to be Data Deficient (Table 10): *Kinosternon durangoense* (Table 7). This turtle is a country endemic that was recognized as a distinct species in 2001 (Serb et al. 2001) by being elevated from a subspecies of *K. flavescens*, but very little information on its biology and conservation status is available to date. Therefore, this appears to be a sound assessment.



No. 13. *Sceloporus samcolemani* Smith and Hall, 1974. Coleman's Bunch Grass Lizard is a Mexican endemic species that ranges from "extreme southeastern Coahuila and southern central Nuevo León" (Watkins-Colwell et al. 1998: 675.2). This individual was seen at Monterreal, in the municipality of Arteaga. Wilson et al. (2013a) gauged its EVS as 15, placing it in the lower portion of the high vulnerability category. Its conservation status has been determined as Least Concern, but this lizard is not listed by SEMARNAT. *Photo by Michael S. Price.*



No. 14. *Uma exsul* Schmidt and Bogert (1947). The Fringe-toed Sand Lizard is a Mexican endemic species occurring in extreme southwestern and south-central Coahuila (Lemos-Espinal and Smith 2007). This individual was located at ca. 2 km SW from Ejido Alejandria, in the municipality of San Pedro. Wilson et al. (2013a) assessed its EVS as 16, placing it in the middle portion of the high vulnerability category. Its conservation status is evaluated as Endangered by IUCN and as endangered (P) by SEMARNAT. *Photo by Marco Antonio Bazán-Tellez.*



No. 15. *Plestiodon dicei* (Ruthven and Gaige, 1933). Dice's Short-nosed Skink is a Mexican endemic occurring from central Nuevo León eastward to central and southern Tamaulipas (Feria-Ortiz et al. 2011). This individual was found in Monterreal, in the municipality of Arteaga. Wilson et al. (2013a) calculated its EVS as 12, placing it in the upper portion of the medium vulnerability category. Its conservation status has not been evaluated by IUCN and this species is not listed by SEMARNAT. *Photo by Elí García Padilla.*



No. 16. *Bogertophis subocularis* (Brown, 1901). The Trans-Pecos Rat Snake is distributed “in southern New Mexico, southwestern Texas, and in northeastern Mexico, from Chihuahua through Coahuila and into Nuevo León and through Durango down to its border with Zacatecas” (Lemos-Espinal et al. 2015: 318). This individual came from Cuatrociénegas, in the municipality of Cuatrociénegas de Carranza. Wilson et al. (2013a) judged its EVS as 14, placing it at the lower edge of the high vulnerability category. Its conservation status is evaluated as Least Concern by IUCN, but it is not listed by SEMARNAT. *Photo by Michael S. Price.*

Twenty-three species (16.4%) in the native Coahuilan herpetofauna remain unevaluated (Table 10). The status of these species is examined further using the EVS system in the following section.

The EVS System. The EVS system, developed originally for use in the conservation assessment of the Honduran herpetofauna (Wilson and McCranie 2004), has been applied subsequently to the entirety of Mexico (Wilson et al. 2013a,b) and to Central America (Johnson et al. 2015b), as well as to various states and groups of states in Mexico (Alvarado-Díaz et al. 2013; Mata-Silva et al. 2015; Johnson et al. 2015a; Terán-Juárez et al. 2016; Woolrich-Piña et al. 2016, 2017; Nevárez-de los Reyes et al. 2016; Cruz-Sáenz et al. 2017; González-Sánchez et al. 2017). Wilson et al. (2013a,b) described the use of this system for the herpetofauna of Mexico; and it is employed here to assess the conservation status of the herpetofauna of Coahuila (see data in Table 7, summarized in Table 11).

The total range of EVS values for the herpetofauna of Coahuila spans the entire theoretical EVS range (3–20). The most frequent values (more than 10 species) are nine (10), 10 (10), 11 (12), 12 (17), 13 (17), 14 (14), 15 (13), and 16 (12). These eight scores were applied to a total of 104 species, or 74.3% of the total number of native species. The average EVS value is 12.1 (1,693/140). When allocated to the three summary categories of low (3–9), medium (10–13), and high (14–20), the species counts for these categories are 32, 56, and 52, respectively.

The lowest EVS value of 3 was applied to three species of anurans (*Rhinella horribilis*, *Smilisca baudinii*, and *Scaphiopus couchii*), which are geographically and ecologically widespread and have the most widespread reproductive mode (eggs and tadpoles in still water). At the other extreme, one species (the trionychid turtle *Apalone atra*) was provided a value of 20, because of its narrow geographic and ecological distribution and its high level of human persecution.

Comparing the results of the IUCN categorization with those of the EVS system in Table 12, indicates that 14 of the 52 high vulnerability species (26.9%) are judged to occupy one of two IUCN threat categories (EN or VU, note that no species are allocated to the CR category). Seven species (five lizards and two turtles) are evaluated as EN, and seven as VU (one anuran, one salamander, two lizards, one snake, and two turtles). These 14 species comprise 10.0% of the 140 native herpetofaunal species in Coahuila. At the opposite extreme, the 32 low vulnerability species comprise 35.1% of the 93 LC species. As in other Mexican Conservation Series studies, the results of the applications of the IUCN and EVS systems do not complement one another.

Twenty-four species remain unassessed using the IUCN system (allocated to the NE category in Table 7). Four of these species are state endemics (*Gerrhonotus mccoysi*, *Sceloporus gadsdeni*, *Scincella kikaapoa*,

and *Apalone atra*), five are country endemics (*Barisia imbricata*, *Holbrookia approximans*, *Plestiodon dicei*, *Lampropeltis leonis*, and *Crotalus morulus*), and the remainder are non-endemics. The range of EVS values for these 24 species is 6–20, which places some of them into each of the three summary categories (Table 13). Four have low EVS scores, ten have medium scores, and ten have high scores. Until such time as IUCN evaluations are available for these species, we suggest that the high EVS species should be placed in one of the three threat categories, perhaps as follows: CR—*Gerrhonotus mccoysi*, *Sceloporus gadsdeni*, *Scincella kikaapoa*, *Lampropeltis leonis*, *Crotalus morulus*, *Apalone atra*; EN—*Barisia imbricata*, *Holbrookia approximans*; VU—*Aspidoscelis marmorata*, *Salvadora deserticola*. We also suggest that the species with EVS of 12 or 13 be placed in the NT category. The remainder of the species with EVS of 6–11 can be allocated to the LC category (Table 13).

As with other studies in the Mexican Conservation Series, this study found that a significantly large number of the Coahuilan herpetofauna members have been allocated by the IUCN to the Least Concern category. The number of such species amounts to 93 (66.4% of the total of 140 species). Given that almost seven of every ten herpetofaunal species in Coahuila is judged Least Concern, it might appear that the state herpetofauna is in relatively good shape with respect to conservation status. In order to ascertain whether such an optimistic view is the case, the 93 species in Table 14 were placed along with the calculations for their respective EVS values. Although one might expect that the LC species would most likely be non-endemic to Mexico, this analysis found that 12 are country endemics, including one salamander, nine lizards, and two snakes, and one lizard is a state endemic (Table 14). The range of EVS values for these 93 species is 3–18, or only slightly less than the entire theoretical range for EVS (3–20). The allocation of the EVS values for the 93 species into the three summary categories demonstrates the following: low (3–9)—27; medium (10–13)—45; and high (14–20)—22. Based on these allocations, we suggest that a more realistic assessment would place the 22 high vulnerability species in one of the three IUCN threat categories, as follows: CR (*Gerrhonotus lugoi*, *Thamnophis exsul*, and *Gopherus berlandieri*); EN (*Coleonyx reticulatus*, *Sceloporus cautus*, *S. couchii*, *S. parvus*, *S. samcolemani*, *Xantusia extorris*, *Lampropeltis mexicana*, *Pantherophis bairdi*, and *Apalone spinifera*); and VU (*Coleonyx brevis*, *Cophosaurus texanus*, *Sceloporus minor*, *Aspidoscelis inornata*, *A. tessellata*, *Bogertophis subocularis*, *Lampropeltis alterna*, *Pituophis deppei*, *Agkistrodon laticinctus*, and *Crotalus pricei*). We also suggest that the 43 medium vulnerability species probably should be placed in the NT category, and that the 27 low vulnerability species could remain in the LC category.

Table 11. Environmental Vulnerability Scores (EVS) for herpetofaunal species in Coahuila, Mexico, arranged by family. Shaded area to the left encompasses low vulnerability scores, and the one to the right high vulnerability scores. Non-native species are excluded.

Families	Number of Species	Environmental Vulnerability Scores																		
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Bufonidae	7	1	—	1	1	1	—	1	1	—	1	—	—	—	—	—	—	—	—	
Craugastoridae	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	
Eleutherodactylidae	3	—	—	—	—	—	—	—	—	1	1	—	—	1	—	—	—	—	—	
Hylidae	4	1	—	—	—	1	—	1	—	—	1	—	—	—	—	—	—	—	—	
Microhylidae	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	
Ranidae	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	
Scaphiopodidae	2	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Subtotals	19	3	—	1	2	3	1	3	1	1	3	—	—	1	—	—	—	—	—	
Ambystomatidae	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	
Plethodontidae	3	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	
Subtotals	4	—	—	—	—	—	—	—	1	—	—	—	—	—	1	1	—	—	—	
Totals	23	3	—	1	2	3	1	3	2	1	3	—	—	1	1	1	1	—	—	
Anguidae	5	—	—	—	—	—	—	—	—	—	—	1	—	1	1	2	—	—	—	
Crotaphytidae	4	—	—	—	—	—	—	—	—	—	1	2	—	—	1	—	—	—	—	
Eublepharidae	2	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	
Phrynosomatidae	29	—	—	—	—	1	—	2	1	2	4	4	4	5	4	2	—	—	—	
Scincidae	3	—	—	—	—	—	—	—	—	1	2	—	—	—	—	—	—	—	—	
Sphenomorphidae	3	—	—	—	—	—	—	—	—	—	1	1	—	—	—	1	—	—	—	
Teiidae	4	—	—	—	—	—	—	1	—	—	—	—	3	—	—	—	—	—	—	
Xantusiidae	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	
Subtotals	51	—	—	—	—	1	—	3	1	3	8	8	8	8	6	5	—	—	—	
Colubridae	29	—	—	1	1	—	2	3	4	3	3	5	4	2	1	—	—	—	—	
Dipsadidae	4	—	1	—	1	—	1	—	—	1	—	—	—	—	—	—	—	—	—	
Elapidae	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	
Leptotyphlopidae	3	—	—	—	—	—	1	—	—	1	—	1	—	—	—	—	—	—	—	
Natricidae	7	—	—	—	—	2	—	—	2	1	—	1	—	—	1	—	—	—	—	
Viperidae	10	—	—	—	—	—	1	1	—	1	2	2	2	—	1	—	—	—	—	
Subtotals	54	—	1	1	2	2	5	4	6	8	5	9	6	2	3	—	—	2	—	
Emydidae	5	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	1	—	—	
Kinosternidae	3	—	—	—	—	—	—	—	1	—	1	—	—	—	1	—	—	—	—	
Testudinidae	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	
Trionychidae	2	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1	
Subtotals	12	—	—	—	—	—	—	—	1	—	1	—	—	2	2	—	2	3	1	
Totals	117	—	1	2	2	3	5	7	8	11	14	17	14	12	11	5	2	3	1	
Sum Totals	140	3	1	2	4	6	6	10	10	12	17	17	14	13	12	6	3	3	1	
Category Totals	140	32										56					52			

Table 12. Comparison of Environmental Vulnerability Scores (EVS) and applicable IUCN categorizations for members of the herpetofauna of Coahuila, Mexico. Non-native species are excluded. No species are allocated to the CR IUCN categories. Shaded area at the top encompasses low vulnerability category scores, and the one at the bottom high vulnerability category scores.

EVS	IUCN Categories						Total
	Endangered	Vulnerable	Near Threatened	Least Concern	Data Deficient	Not Evaluated	
3	—	—	—	3	—	—	3
4	—	—	—	1	—	—	1
5	—	—	—	2	—	—	2
6	—	—	—	3	—	1	4
7	—	—	—	6	—	—	6
8	—	—	—	4	—	2	6
9	—	—	1	8	—	1	10
10	—	—	—	10	—	—	10
11	—	—	—	10	—	2	12
12	—	1	—	12	—	4	17
13	—	1	—	12	—	4	17
14	—	—	1	10	—	3	14
15	1	1	1	9	—	1	13
16	3	1	3	2	1	2	12
17	1	1	1	—	—	3	6
18	—	1	1	1	—	—	3
19	2	1	—	—	—	—	3
20		—	—	—	—	1	1
Totals	7	7	8	93	1	24	140

Table 13. Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Coahuila, Mexico, currently not evaluated (NE) by the IUCN. Non-native taxa are not included. * = country endemic species; ** = state endemic species.

Taxa	Environmental Vulnerability Score			
	Geographic Distribution	Ecological Distribution	Reproductive Mode/Degree of Persecution	Total Score
<i>Acris blanchardi</i>	3	8	1	12
<i>Barisia ciliaris</i> *	5	7	3	15
<i>Gerrhonotus mccoysi</i> **	6	8	3	17
<i>Holbrookia approximans</i> *	5	6	3	14
<i>Sceloporus cowlesi</i>	4	6	3	13
<i>Sceloporus cyanogenys</i>	4	6	3	13
<i>Sceloporus gadsdeni</i> **	6	8	3	17
<i>Sceloporus marmoratus</i>	2	6	3	11
<i>Plestiodon dicei</i> *	5	4	3	12
<i>Scincella kikaapoa</i> **	6	8	3	17
<i>Aspidoscelis marmorata</i>	4	7	3	14
<i>Lampropeltis annulata</i>	4	3	5	12
<i>Lampropeltis gentilis</i>	3	1	5	9
<i>Lampropeltis leonis</i> *	5	6	5	16
<i>Lampropeltis splendida</i>	4	5	3	12
<i>Salvadora deserticola</i>	4	6	4	14
<i>Sonora episcopa</i>	3	7	3	13
<i>Heterodon kennerlyi</i>	3	4	4	11
<i>Hypsiglena jani</i>	1	3	2	6
<i>Leptodeira septentrionalis</i>	2	2	4	8
<i>Rena segrega</i>	4	3	1	8
<i>Crotalus morulus</i>	5	6	5	16
<i>Crotalus ornatus</i>	4	4	5	13
<i>Apalone atra</i> **	6	8	6	20

Table 14. Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Coahuila, Mexico, assigned to the IUCN Least Concern category. Non-native taxa are not included. * = country endemic species; ** = state endemic species.

Taxa	Environmental Vulnerability Score			
	Geographic Distribution	Ecological Distribution	Reproductive Mode/Degree of Persecution	Total Score
<i>Anaxyrus cognatus</i>	3	5	1	9
<i>Anaxyrus debilis</i>	1	5	1	7
<i>Anaxyrus punctatus</i>	1	3	1	5
<i>Anaxyrus speciosus</i>	4	7	1	12
<i>Anaxyrus woodhousii</i>	3	6	1	10
<i>Incilius nebulifer</i>	1	4	1	6
<i>Rhinella horribilis</i>	1	1	1	3
<i>Craugastor augusti</i>	2	2	4	8
<i>Eleutherodactylus cystignathoides</i>	2	6	4	12
<i>Eleutherodactylus guttilatus</i>	2	5	4	11
<i>Dryophytes arenicolor</i>	2	4	1	7
<i>Smilisca baudinii</i>	1	1	1	3
<i>Gastrophryne olivacea</i>	3	5	1	9
<i>Lithobates berlandieri</i>	4	2	1	7
<i>Scaphiopus couchii</i>	1	1	1	3
<i>Spea multiplicata</i>	1	4	1	6
<i>Ambystoma velasci</i> *	5	4	1	10
<i>Gerrhonotus infernalis</i>	5	5	3	13
<i>Gerrhonotus lugoi</i> **	5	8	3	16
<i>Crotaphytus collaris</i>	3	7	3	13
<i>Crotaphytus wislizenii</i>	3	7	3	13
<i>Coleonyx brevis</i>	4	6	4	14
<i>Coleonyx reticulatus</i>	4	7	4	15
<i>Cophosaurus texanus</i>	4	7	3	14
<i>Phrynosoma cornutum</i>	1	7	3	11
<i>Phrynosoma modestum</i>	4	5	3	12
<i>Phrynosoma orbiculare</i> *	5	4	3	12
<i>Sceloporus cautus</i> *	5	7	3	15
<i>Sceloporus couchii</i> *	5	7	3	15
<i>Sceloporus grammicus</i>	2	4	3	9
<i>Sceloporus magister</i>	1	5	3	9
<i>Sceloporus merriami</i>	4	6	3	13
<i>Sceloporus minor</i> *	5	6	3	14
<i>Sceloporus olivaceus</i>	4	6	3	13
<i>Sceloporus parvus</i> *	5	7	3	15
<i>Sceloporus poinsettii</i>	4	5	3	12
<i>Sceloporus samcolemani</i> *	5	7	3	15
<i>Sceloporus spinosus</i> *	5	4	3	12
<i>Urosaurus ornatus</i>	2	5	3	10
<i>Uta stansburiana</i>	3	1	3	7
<i>Plestiodon obsoletus</i>	3	5	3	11
<i>Plestiodon tetragrammus</i>	4	5	3	12
<i>Scincella lateralis</i>	3	7	3	13
<i>Scincella silvicola</i> *	5	4	3	12
<i>Aspidoscelis gularis</i>	2	4	3	9
<i>Aspidoscelis inornata</i>	4	7	3	14
<i>Aspidoscelis tessellata</i>	4	7	3	14
<i>Xantusia extorris</i> *	5	7	3	15
<i>Arizona elegans</i>	1	1	3	5
<i>Bogertophis subocularis</i>	4	7	3	14
<i>Coluber constrictor</i>	1	6	3	10

Table 14 (continued). Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Coahuila, Mexico, assigned to the IUCN Least Concern category. Non-native taxa are not included. * = country endemic species; ** = state endemic species.

Taxa	Environmental Vulnerability Score			
	Geographic Distribution	Ecological Distribution	Reproductive Mode/Degree of Persecution	Total Score
<i>Gyalopion canum</i>	4	3	2	9
<i>Lampropeltis alterna</i>	4	7	3	14
<i>Masticophis flagellum</i>	1	3	4	8
<i>Masticophis schotti</i>	4	5	4	13
<i>Masticophis taeniatus</i>	1	5	4	10
<i>Opheodrys aestivus</i>	3	7	3	13
<i>Pantherophis bairdi</i>	4	7	4	15
<i>Pantherophis emoryi</i>	3	6	4	13
<i>Pituophis catenifer</i>	4	1	4	9
<i>Pituophis deppei</i> *	5	5	4	14
<i>Rhinocheilus lecontei</i>	1	3	4	8
<i>Salvadora grahamiae</i>	4	2	4	10
<i>Tantilla atriceps</i>	2	7	2	11
<i>Tantilla cucullata</i>	4	6	2	12
<i>Tantilla gracilis</i>	3	8	2	13
<i>Tantilla hobartsmithi</i>	3	6	2	11
<i>Tantilla nigriceps</i>	3	6	2	11
<i>Tantilla wilcoxi</i>	2	6	2	10
<i>Trimorphodon wilkinsonii</i>	4	7	4	15
<i>Diadophis punctatus</i>	1	1	2	4
<i>Micrurus tener</i>	1	5	5	11
<i>Rena dissecta</i>	4	6	1	11
<i>Rena dulcis</i>	4	8	1	13
<i>Nerodia erythrogaster</i>	3	4	4	11
<i>Nerodia rhombifer</i>	1	5	4	10
<i>Thamnophis cyrtopsis</i>	2	1	4	7
<i>Thamnophis exsul</i> *	5	7	4	16
<i>Thamnophis marcianus</i>	1	5	4	10
<i>Thamnophis proximus</i>	1	2	4	7
<i>Agkistrodon contortrix</i>	3	6	5	14
<i>Crotalus atrox</i>	1	3	5	9
<i>Crotalus lepidus</i>	2	5	5	12
<i>Crotalus molossus</i>	2	1	5	8
<i>Crotalus pricei</i>	2	7	5	14
<i>Crotalus scutulatus</i>	2	4	5	11
<i>Crotalus viridis</i>	1	6	5	12
<i>Sistrurus tergeminus</i>	3	5	5	13
<i>Kinosternon flavescens</i>	3	6	3	12
<i>Kinosternon hirtipes</i>	2	5	3	10
<i>Gopherus berlandieri</i>	4	8	6	18
<i>Apalone spinifera</i>	3	6	6	15

Relative Herpetofaunal Priority

Johnson et al. (2015a) developed the concept of Relative Herpetofaunal Priority (RHP), a simple means for measuring the relative importance of the herpetofaunal species found in any geographic entity (e.g., a state or a physiographic region). Determining the RHP involves the use of two methods, i.e., (1) calculating the proportion

of state and country endemics relative to the entire physiographic regional herpetofauna, and (2) computing the absolute number of EVS high category species in each physiographic regional herpetofauna.

Here, two tables have been constructed to ascertain the RHP values for the Coahuilan herpetofauna, one for the endemicity values (Table 15) and the other for the high category EVS values (Table 16). The data in Table

Table 15. Numbers of herpetofaunal species in four distributional categories among the 10 physiographic provinces of Coahuila, Mexico. Rank determined by adding state and country endemics.

Physiographic Province	Non-endemics	Country endemics	State endemics	Non-natives	Total	Rank Order
Bolsón de Mapimi	36	5	2	2	45	4
Llanuras y Sierras Volcánicas	42	1	—	1	44	7
Laguna de Mayrán	35	1	1	1	38	6
Sierras y Llanuras Coahuilenses	80	3	5	3	91	3
Serranía del Burro	43	1	—	1	45	7
Sierra de la Paila	37	2	—	1	40	6
Pliegues Saltillo Parras	41	6	—	2	49	5
Sierras Transversales	32	13	—	1	46	2
Gran Sierra Plegada	32	18	—	1	51	1
Llanuras de Coahuila y Nuevo León	50	—	1	2	53	7

15 demonstrate that the highest amount of endemism is found in the Gran Sierra Plegada, only a small portion of which is located in Coahuila (Fig. 1); thus, this region occupies rank number 1 using this measure. Of the 51 species recorded in this region, 18 are country endemics (35.3%). Nevárez-de los Reyes et al. (2016) found a similar situation prevailing in the neighboring state of Nuevo León, in which 33 of 87 species (37.9%) in this same region consist of country and state endemics. Rank number 2 is occupied by the Sierras Transversales located along the southern border of Coahuila, in which 13 of 46 species (28.3%) are country endemics. The remaining eight physiographic regions occupy ranks 3 through 7, given that several of these regions share the same rank with other regions (Table 15). One region (Sierras y Llanuras Coahuilenses) occupies rank 3, and contains eight country and state endemics. Only one region occupies rank 4, which is the Bolsón de Mapimí, with five country endemics and two state endemics. One region (Pliegues Saltillo Parras) lies at rank 5, with six country endemics. Two regions (Laguna de Mayrán and Sierra de la Paila) occupy rank 6, with two endemics each. Finally, there are three regions (Llanuras y Sierras Volcánicas, Serranías del Burro, and Llanuras de Coahuila y Nuevo León) that occupy rank 7, each with a single endemic species.

The numbers of species for each of the 10 physiographic regions are placed into the three EVS

categories (low, medium, and high) in Table 16. These data indicate that the greatest number of high vulnerability species (22 of 88, 25.0%) is found in the Sierras y Llanuras Coahuilenses in the central portion of the state (Fig. 1), thus this region occupies rank number 1. The next highest number (17 of 50 species, or 34.0%) is found in the Gran Sierra Plegada, which is located in the southeastern corner of the state (Fig. 1) and occupies rank number 2. The 3rd rank is occupied by the Sierras Transversales, with 14 high-vulnerability species out of 46 (30.4%). The numbers of high EVS species in the remaining seven regions range from seven to 13.

The rankings obtained by using these two RHP methods are not identical, but at least the regions occupying ranks 1 through 3 are the same three regions in each case, as follows (endemic species ranking followed by high vulnerability ranking):

- Gran Sierra Plegada (1, 2)
- Sierras Transversales (2, 3)
- Sierras y Llanuras Coahuilenses (3, 1)

The results of this RHP analysis clearly show that the most important region in the state is the Gran Sierra Plegada, occupying rank 1 for endemic species and rank 2 for high vulnerability species. This finding is interesting, given the small amount of this physiographic region situated in Coahuila (2,178 km², as noted above),

Table 16. Number of herpetofaunal species in the three EVS categories among the 10 physiographic regions of Coahuila, Mexico. Rank determined by the relative number of high EVS species. Non-native species are excluded.

Physiographic Province	Low	Medium	High	Total	Rank Order
Bolsón de Mapimi	16	15	12	43	5
Llanuras y Sierras Volcánicas	18	16	9	43	6
Laguna de Mayrán	16	14	7	37	7
Sierras y Llanuras Coahuilenses	28	38	22	88	1
Serranía del Burro	19	16	9	44	6
Sierra de la Paila	17	15	7	39	7
Pliegues Saltillo Parras	17	17	13	47	4
Sierras Transversales	14	18	14	46	3
Gran Sierra Plegada	16	17	17	50	2
Llanuras de Coahuila y Nuevo León	16	27	7	50	7



No. 17. *Rhinocheilus lecontei* Baird and Girard, 1853. The Long-nosed Snake occurs from “California to Kansas, excluding much of the Great Basin and the Rocky Mountains, southward to Baja California and Nayarit and, east of the Sierra Madre [Occidental], to the southern limited of the Chihuahua Desert” (Lemos-Espinal et al. 2015: 371). This individual was located at ca. 4 km east of Nava, in the municipality of Nava. Wilson et al. (2013a) assessed its EVS as 8, placing it in the upper portion of the low vulnerability category. Its conservation status is judged as Least Concern by IUCN, but it is not listed by SEMARNAT. *Photo by Marco Antonio Bazán-Tellez.*



No. 18. *Tantilla atriceps* (Günther, 1895). The Mexican Black-headed Snake is distributed from “extreme southern Texas southward through central Coahuila to extreme northeastern Durango and northern Tamaulipas and San Luis Potosí” (Lemos-Espinal et al. 2015: 382). This individual came from Rancho La Boca, on the border of the municipalities of Bustamante and Mina. Wilson et al. (2013a) calculated its EVS as 11, placing it in the lower portion of the medium vulnerability category. Its conservation status has been considered as Least Concern by IUCN, and as a threatened species (A) by SEMARNAT. *Photo by Michael S. Price.*



No. 19. *Micrurus tener* (Baird and Girard, 1853). The Texas Coralsnake occurs “from the Mississippi River westward into Texas, in the United States, and in Mexico, from Tamaulipas south to Veracruz...” (Lemos-Espinal and Dixon 2013: 240). This individual came from ca. 16 km east of Nava, in the municipality of Nava. Wilson et al. (2013a) calculated its EVS as 11, placing it in the middle of the medium vulnerability category. Its conservation status has been determined as Least Concern by IUCN, and this species is not listed by SEMARNAT. *Photo by Marco Antonio Bazán-Tellez.*



No. 20. *Crotalus atrox* Baird and Girard, 1853. The Western Diamondback Rattlesnake occupies “much of the southwestern USA south to northeastern Baja California, Sonora and northern Sinaloa, and east of the Sierra Madre Occidental from Chihuahua east to Tamaulipas and south to Hidalgo and Veracruz” (Rorabaugh and Lemos-Espinal 2016: 573). This individual was found at ca. 1 km NW from Ejido Mieleras, in the municipality of Viesca. Wilson et al. (2013a) determined its EVS as 9, placing it at the upper limit of the low vulnerability category. Its conservation status is judged as Least Concern by IUCN and as a species of special protection (Pr) by SEMARNAT. *Photo by Marco Antonio Bazán-Tellez.*

although it has considerably more area outside the state. The two other regions indicated above (Sierras Transversales and Sierras y Llanuras Coahuilenses) are considerably larger. The Gran Sierra Plegada supports 18 country endemics (Table 15) and 17 high vulnerability species (Table 16). The Sierras Transversales contains 13 country endemics (Table 15) and 14 high vulnerability species (Table 16); the respective figures for the Sierras y Llanuras Coahuilenses are eight endemic species and 22 high vulnerability species. The level of protection provided for these various species is indicated in the following section on protected areas.

Protected Areas in Coahuila

A system of formally protected areas is integral to any effort to protect any portion of the planetary biota from the principal anthropogenic impacts brought about by habitat degradation and destruction. Ostensibly, such a system should incorporate as much of the environmental diversity that exists within the target region (in this case, the state of Coahuila) and as large of a portion of that patrimony as is feasible within existing economic confines. In an effort to determine how these concerns have been addressed in Coahuila, information on the protected areas in the state has been collated and presented in Table 17.

The data in Table 17 indicate that of the 19 protected areas listed, eight are federal reserves, four are federal/private reserves, three are state reserves, three are state/private reserves, and one is a municipal reserve. The eight areas administered at the federal level include one biosphere reserve, one national park, three floral and faunal protection areas, two resource protection areas, and one national monument.

These 19 areas in Coahuila have been established over the 100 years from 1915 to 2015. Thirteen of these 19 areas have been in existence only since the turn of the century or thereafter, while three were established in the decade of the 1990s, two in the decade of the 1940s, and one in 1915. Thus, it remains to be seen in the analysis of the remainder of the data in Table 17 exactly what has been accomplished in these areas, especially since 68.4% of them (13/19) have existed for fewer than 17 years.

The areas of coverage of these protected areas range broadly from as low as 38 ha to as high as 1,519,385 ha. Interestingly, the largest of the 19 areas is the Área de Protección de Recursos Naturales Cuenca Alimentadora del Distrito Nacional de Riego 04 Don Martín, established in 1915, which is the one with the longest existence. The total coverage of these areas is 2,717,443 ha or approximately 27,174 km², which is 17.9% of the area of Coahuila. Portions of these 19 areas are located in 23 of the 38 municipalities (60.5%).

This system of protected areas in the state contains representatives of eight of the ten physiographic regions, including the Bolsón de Mapimí (one of 20 areas),

Llanuras y Sierras Volcánicas (two), Laguna de Mayrán (one), Sierras y Llanuras Coahuilenses (eight), Serranías del Burro (two), Sierra Transversales (four), Gran Sierra Plegada (four), and Llanuras de Coahuila y Nuevo León (two). The two regions with no representation are the Sierra de la Paila and the Pliegues Saltillo Parras. It is no doubt fortuitous that the three physiographic regions with the greatest amount of representation in the protected areas system are the regions having the great herpetofaunal significance, i.e., the Gran Sierra Plegada, Sierras Transversales, and Sierras y Llanuras Coahuilenses. Unfortunately, however, of the protected areas for which such information is available, all are known to be occupied to some extent by landowners.

Management plans are known to be available for only five of the federal protected areas, which points out the great need for completion of such plans for the remainder of the areas. Even less well represented are herpetofaunal surveys, of which there are only three completed and three in the process of completion. Completion of the remainder of the surveys needs to be undertaken as soon as possible.

In general, systems of protected areas are established without consideration of the conservation needs of the herpetofauna, so it is not surprising that this need is still outstanding in Coahuila. The good news is that the physiographic regions that are herpetofaunally most important, based on our RHP analyses, are those best supplied with protected areas. The bad news is that sizable proportions of those areas are occupied by landowners, have no management plans designed, and have been subject to no herpetofaunal surveys. Thus, at present, there is no chance of answering any of the more pressing questions about the state of population sustainability of the component species of Coahuila's herpetofauna. So, redressing these inadequacies in the design and implementation of the protected areas system has to be undertaken so that the ability of the current system of protected areas to provide for perpetual protection of the state's herpetofauna can be assessed and strengthened.

Here, the expected herpetofaunal content of the 19 protected areas in Coahuila is catalogued by compiling the species known to occupy the physiographic regions represented in each of these regions. We employed this means since too few herpetofaunal surveys have been undertaken in these areas to date. Thus, ground-based field surveys will be necessary to provide the empirical data required to substantiate the actual herpetofaunal content of the state's protected areas. Using this approach, the results are shown in Table 18, and summarized in Table 19.

Of the 143 species recorded in Coahuila, 120 (83.9%) are expected to be found in one or more of the 19 protected areas (Table 19). The number of species expected to occur in each area ranges from three in the Parque Estatal Bosque Urbano Ejército Mexicano to 84 in the Área de Protección de Flora y Fauna Cuatro Ciénegas.

Table 17. Characteristics of the Natural Protected Areas (ANPs) in Coahuila, Mexico. Abbreviations for the facilities in the ANPs are as follows: A = administrative services; R = park guards; S = systems of pathways; and V = facilities for visitors. Information in part from: <http://www.sema.gob.mx/SRN-CONSER-ANP-LISTADO.html>; <http://www.conanp.gob.mx/movil/programas.php>; http://sig.conanp.gob.mx/website/pagsig/anps_decretadas/lista_anps.pdf; <https://www.gob.mx/conanp/acciones-y-programas/areas-destinadas-voluntariamente-a-la-conservacion>; accessed 6 December 2017.

Name	Category	Date of decree	Area (ha)	Municipality	Jurisdiction	Physiographic Region	Facilities Available	Occupied by Landowners	Herpetofaunal Survey Completed	Management Plan Available
Río Bravo del Norte	Monumento Natural	21 Oct 2009	2,175	Acuña, Ocampo	Federal	SLC, SDB	A, R	Yes	No	Yes
Maderas del Carmen	Área de Protección de Flora y Fauna	07 Nov 1994	208,381	Acuña, Múzquiz, Ocampo	Federal	SLC	A, R	Yes	In process	Yes
Ocampo	Área de Protección de Flora y Fauna	05 Jun 2009	344,238	Ocampo	Federal	SLC	A, R	Yes	No	Yes
Cuatro Ciénegas	Área de Protección de Flora y Fauna	07 Nov 1994	84,347	Cuatro Ciénegas	Federal	SLC	A, R, S, V	Yes	Yes	Yes
Mapimí	Reserva de la Biósfera	27 Nov 2000	86,606	Francisco I. Madero, Sierra Mojada	Federal	BDM		Yes	Yes	Yes
Los Novillos	Parque Nacional	18 Jun 1940	38	Acuña	Federal	LCN	A, R, S, V	Yes	No	No
Cuenca Alimentadora del Distrito Nacional de Riego 04 Don Martín	Área de Protección de Recursos Naturales	16 May 1915	1,519,385	Acuña, Candela, Castaños, Cuatro Ciénegas, Lamadrid, Juárez, Monclova, Múzquiz, Nadores, Ocampo, Sabinas, Sacramento, San Buenaventura, San Juan de Sabinas, Zaragoza	Federal	SLC, SDB, LCN	A, R	Yes	No	No
Distrito Nacional de Riego 026 Bajo Río San Juan.	Área de Protección de Recursos Naturales	03 Aug 1949 (07 Nov 2002)	197,157 (combined total in Coahuila and Nuevo León)	Arteaga, Ramos Arizpe	Federal	GSP		Yes	No	No
Rancho Media Luna	Área Destinada Voluntariamente a la Conservación	2005	17,181	Ocampo	Federal/Private	SLC, LSV	N.A.	N.A.	N.A.	N.A.
Rancho La Puerta	Área Destinada Voluntariamente a la Conservación	2015	1,443	Saltillo	Federal/Private	GSP	N.A.	N.A.	N.A.	N.A.

Table 17 (continued). Characteristics of the Natural Protected Areas (ANPs) in Coahuila, Mexico. Abbreviations for the facilities in the ANPs are as follows: A = administrative services; R = park guards; S = systems of pathways; and V = facilities for visitors. Information in part from: <http://www.sema.gob.mx/SRN-CONSER-ANP-LISTADO.html>; <http://www.conanp.gob.mx/movil/programas.php>; http://sig.conanp.gob.mx/website/pagsig/anps_decretadas/lista_anps.pdf; <https://www.gob.mx/conanp/acciones-y-programas/areas-destinadas-voluntariamente-a-la-conservacion>; accessed 6 December 2017.

Name	Category	Date of decree	Area (ha)	Municipality	Jurisdiction	Physiographic Region	Facilities Available	Occupied by Landowners	Herpetofaunal Survey Completed	Management Plan Available
Tierra Silvestre Cañón del Diablo	Área Destinada Voluntariamente a la Conservación	2009	22,378	Acuña	Federal/Private	SLC	N.A.	N.A.	N.A.	N.A.
Sierra San Vicente	Área Destinada Voluntariamente a la Conservación	2007	15,247	Ocampo	Federal/Private	SLC, LSV	N.A.	N.A.	N.A.	N.A.
Sierra de Zapalinamé	Zona Sujeta a Conservación Ecológica	15 Oct 1996(1ª) 4 Feb 1997 (2ª)	25,680	Arteaga, Saltillo,	State	GSP	N.A.	N.A.	In process	N.A.
Zapalinamé	Zona de Restauración	08 Jun 2007	1,827 (1,707.75)	Arteaga, Saltillo, Arteaga	State	GSP	N.A.	N.A.	In process	N.A.
Bosque Urbano Ejército Mexicano	Parque Estatal	22 Nov 2013	51	Saltillo	State	STR	N.A.	N.A.	No	N.A.
El “Tulillo”	Área Destinada a la Preservación de los Ecosistemas y su Biodiversidad de Iniciativa Voluntaria	26 Nov 2013	66	General Cepeda	State/Private	STR	N.A.	N.A.	No	N.A.
Villa Bilbao	Área Destinada a la Preservación de los Ecosistemas y su Biodiversidad de Iniciativa Voluntaria	17 Jun 2014	21,001	Viesca	State/Private	LDM	N.A.	N.A.	No	N.A.
Tomás Garrido	Área Destinada a la Preservación de los Ecosistemas y su Biodiversidad de Iniciativa Voluntaria	17 Jun 2014	16,206	Viesca	State/Private	STR	N.A.	N.A.	No	N.A.
Sierra y Cañón de Jimulco	Zona Natural Protegida	27 Jun 2003	48,649	Torreón	Municipal	STR	N.A.	N.A.	Yes	N.A.

Table 18. Distribution of herpetofaunal species in Natural Protected Areas of Coahuila, Mexico, based on estimated inclusions. Abbreviations are as follows: * = species endemic to Mexico; ** = species endemic to Coahuila; and *** = non-native species.

Taxa	Natural Protected Area																		
	Río Bravo del Norte	Maderas del Carmen	Ocampo	Cuatro Ciénegas	Mapimí	Los Novillos	Don Martín	Bajo Río San Juan.	Rancho Media Luna	Rancho La Puerta	Cañón del Diablo	Sierra San Vicente	Sierra de Zapalinamé	Zapalinamé	BU Ejército Mexicano	El “Tulillo”	Villa Bilbao	Tomás Garrido	Sierra y Cañón de Jimulco
Anura (16 species)																			
Bufonidae (7 species)																			
<i>Anaxyrus cognatus</i>	+	+	+	+	+		+		+		+	+					+		
<i>Anaxyrus debilis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
<i>Anaxyrus punctatus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
<i>Anaxyrus speciosus</i>	+	+	+	+		+	+		+		+	+							
<i>Anaxyrus woodhousii</i>					+														
<i>Incilius nebulifer</i>	+	+	+	+		+	+		+		+	+							
<i>Rhinella horribilis</i>	+	+	+	+		+	+		+		+	+							
Craugastoridae (1 species)																			
<i>Craugastor augusti</i>				+															
Eleutherodactylidae (2 species)																			
<i>Eleutherodactylus cysthignathoides</i>						+	+												
<i>Eleutherodactylus guttilatus</i>	+	+	+	+			+		+		+	+							
Hylidae (1 species)																			
<i>Dryophytes arenicolor</i>	+	+	+	+					+		+	+							
Microhylidae (1 species)																			
<i>Gastrophryne olivacea</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+		
Ranidae (2 species)																			
<i>Lithobates berlandieri</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
<i>Lithobates catesbeianus</i> ***	+	+																	
Scaphiopodidae (2 species)																			
<i>Scaphiopus couchii</i>	+	+	+	+	+	+	+	+	+	+	+	+				+	+	+	+
<i>Spea multiplicata</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
Squamata (93 species)																			
Anguidae (4 species)																			
<i>Gerrhonotus infernalis</i>	+	+	+	+	+		+	+	+	+	+	+	+	+		+	+	+	+
<i>Gerrhonotus lugoi</i> **				+															
<i>Gerrhonotus mccoysi</i> **				+															
<i>Gerrhonotus parvus</i> *													+	+					
Crotaphytidae (4 species)																			
<i>Crotaphytus antiquus</i> **																	+		

The herpetofauna of Coahuila, Mexico

Table 18 (continued). Distribution of herpetofaunal species in Natural Protected Areas of Coahuila, Mexico, based on estimated inclusions. Abbreviations are as follows: * = species endemic to Mexico; ** = species endemic to Coahuila; and *** = non-native species.

Taxa	Natural Protected Area																		
	Río Bravo del Norte	Maderas del Carmen	Ocampo	Cuatro Ciénegas	Mapimí	Los Novillos	Don Martín	Bajo Río San Juan.	Rancho Media Luna	Rancho La Puerta	Cañón del Diablo	Sierra San Vicente	Sierra de Zapalinamé	Zapalinamé	BU Ejército Mexicano	El “Tulillo”	Villa Bilbao	Tomás Garrido	Sierra y Cañón de Jimulco
<i>Crotaphytus reticulatus</i>							+												
<i>Gambelia wislizenii</i>	+	+	+	+			+		+		+	+							
Eublepharidae (2 species)																			
<i>Coleonyx brevis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
<i>Coleonyx reticulatus</i>		+		+															
Gekkonidae (1 species)																			
<i>Hemidactylus turcicus</i> ***	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phrynosomatidae (22 species)																			
<i>Cophosaurus texanus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
<i>Holbrookia approximans</i> *			+	+	+														+
<i>Phrynosoma cornutum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
<i>Phrynosoma modestum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
<i>Sceloporus cautus</i> *																+		+	+
<i>Sceloporus couchii</i> *			+	+															+
<i>Sceloporus cowlesi</i>	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Sceloporus cyanogenys</i>	+						+												
<i>Sceloporus grammicus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
<i>Sceloporus maculosus</i> *					+														
<i>Sceloporus magister</i>	+	+	+	+			+		+		+	+							
<i>Sceloporus merriami</i>	+	+	+	+			+		+		+	+				+		+	+
<i>Sceloporus olivaceus</i>	+	+	+	+		+	+		+		+	+							
<i>Sceloporus ornatus</i> *													+	+					
<i>Sceloporus parvus</i> *													+	+					
<i>Sceloporus poinsetti</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
<i>Sceloporus samcolemani</i> *																+		+	+
<i>Sceloporus spinosus</i> *													+	+					
<i>Uma exsul</i> **					+														
<i>Uma paraphygas</i> *					+														
<i>Urosaurus ornatus</i>	+	+																	
<i>Uta stansburiana</i>		+	+	+	+		+		+	+	+	+				+	+	+	+
Scincidae (3 species)																			
<i>Plestiodon dicei</i> *								+											
<i>Plestiodon obsoletus</i>	+	+	+	+	+	+	+		+		+	+				+	+	+	+
<i>Plestiodon tetragrammus</i>	+	+	+	+		+	+		+		+	+							
Sphenomorphidae (2 species)																			

Table 18 (continued). Distribution of herpetofaunal species in Natural Protected Areas of Coahuila, Mexico, based on estimated inclusions. Abbreviations are as follows: * = species endemic to Mexico; ** = species endemic to Coahuila; and *** = non-native species.

Taxa	Natural Protected Area														
	Río Bravo del Norte	Maderas del Carmen	Ocampo	Cuatro Ciénegas	Mapimí	Los Novillos	Don Martín	Bajo Río San Juan.	Rancho Media Luna	Rancho La Puerta	Cañón del Diablo	Sierra San Vicente	Sierra de Zapalinamé	Zapalinamé	BU Ejército Mexicano
<i>Scincella lateralis</i>		+													
Teiidae (4 species)															
<i>Aspidoscelis gularis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Aspidoscelis inornata</i>		+	+	+	+		+	+	+	+	+	+	+		+
<i>Aspidoscelis marmorata</i>	+	+	+	+	+		+		+		+	+			+
<i>Aspidoscelis tessellata</i>	+	+	+	+			+		+		+	+			
Xantusiidae (1 species)															
<i>Xantusia extorris</i> *					+										
Colubridae (26 species)															
<i>Arizona elegans</i>	+	+	+	+	+	+	+		+		+	+			+
<i>Bogertophis subocularis</i>	+	+	+	+	+		+		+		+	+			+
<i>Coluber constrictor</i>	+	+	+	+			+		+		+	+			
<i>Drymarchon melanurus</i>	+	+	+	+		+	+		+		+	+			
<i>Gyalopion canum</i>	+	+	+	+		+	+		+		+	+			
<i>Lampropeltis alterna</i>	+	+	+	+			+		+		+	+			
<i>Lampropeltis annulata</i>	+	+	+	+		+	+		+		+	+			
<i>Lampropeltis leonis</i> *								+					+	+	
<i>Lampropeltis splendida</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Masticophis flagellum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Masticophis schotti</i>						+	+								
<i>Masticophis taeniatus</i>	+	+	+	+	+		+		+		+	+			+
<i>Opheodrys aestivus</i>	+	+	+	+			+		+		+	+			
<i>Pantherophis bairdi</i>	+	+	+	+			+		+		+	+			
<i>Pantherophis emoryi</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Pituophis catenifer</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Pituophis deppei</i> *								+		+			+	+	+
<i>Rhinocheilus lecontei</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Salvadora deserticola</i>	+	+	+	+			+		+		+	+			
<i>Salvadora grahamiae</i>	+	+	+	+			+		+		+	+			
<i>Sonora episcopa</i>	+	+	+	+	+	+	+		+		+	+			+
<i>Tantilla atriceps</i>	+	+	+	+			+		+		+	+			
<i>Tantilla gracilis</i>						+	+								
<i>Tantilla hobartsmithi</i>	+	+	+	+			+		+		+	+			
<i>Tantilla nigriceps</i>						+	+								

The herpetofauna of Coahuila, Mexico

Table 18 (continued). Distribution of herpetofaunal species in Natural Protected Areas of Coahuila, Mexico, based on estimated inclusions. Abbreviations are as follows: * = species endemic to Mexico; ** = species endemic to Coahuila; and *** = non-native species.

Taxa	Natural Protected Area														
	Río Bravo del Norte	Maderas del Carmen	Ocampo	Cuatro Ciénegas	Mapimí	Los Novillos	Don Martín	Bajo Río San Juan.	Rancho Media Luna	Rancho La Puerta	Cañón del Diablo	Sierra San Vicente	Sierra de Zapalinamé	Zapalinamé	BU Ejército Mexicano
Dipsadidae (4 species)															
<i>Diadophis punctatus</i>	+	+	+	+			+		+		+	+			
<i>Heterodon kennerlyi</i>	+	+	+	+	+	+	+		+		+	+			+
<i>Hypsiglena jani</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Leptodeira septentrionalis</i>	+	+	+	+			+		+		+	+			
Elapidae (1 species)															
<i>Micrurus tener</i>	+	+	+	+		+	+	+	+	+	+	+	+	+	
Leptotyphlopidae (3 species)															
<i>Rena dissecta</i>	+	+	+	+			+		+		+	+			
<i>Rena dulcis</i>	+	+	+	+		+	+		+		+	+			
<i>Rena segrega</i>	+	+	+	+			+		+		+	+			
Natricidae (7 species)															
<i>Nerodia erythrogaster</i>	+	+	+	+		+	+		+		+	+			
<i>Nerodia rhombifer</i>						+	+								
<i>Storeria hidalgoensis</i> *								+		+			+	+	
<i>Thamnophis cyrtopsis</i>	+	+	+	+			+		+		+	+			
<i>Thamnophis exsul</i> *								+		+			+	+	
<i>Thamnophis marcianus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Thamnophis proximus</i>	+	+	+	+		+	+		+		+	+			
Viperidae (9 species)															
<i>Agkistrodon contortrix</i>	+	+	+	+			+		+		+	+			
<i>Crotalus atrox</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Crotalus lepidus</i>	+	+	+	+			+	+	+	+	+	+	+	+	
<i>Crotalus molossus</i>								+		+			+	+	+
<i>Crotalus morulus</i> *								+		+			+	+	
<i>Crotalus ornatus</i>	+	+	+	+			+		+		+	+			
<i>Crotalus pricei</i>								+		+			+	+	
<i>Crotalus scutulatus</i>	+	+	+	+	+										+
<i>Sistrurus tergeminus</i>				+											
Testudines (11 species)															
Emydidae (5 species)															
<i>Pseudemys gorzugi</i>	+						+								
<i>Terrapene coahuila</i> **				+											
<i>Trachemys gaigeae</i>		+													
<i>Trachemys scripta</i> ***	+	+					+								

Table 18 (continued). Distribution of herpetofaunal species in Natural Protected Areas of Coahuila, Mexico, based on estimated inclusions. Abbreviations are as follows: * = species endemic to Mexico; ** = species endemic to Coahuila; and *** = non-native species.

Taxa	Natural Protected Area																		
	Río Bravo del Norte	Maderas del Carmen	Ocampo	Cuatro Ciénegas	Mapimí	Los Novillos	Don Martín	Bajo Río San Juan.	Rancho Media Luna	Rancho La Puerta	Cañón del Diablo	Sierra San Vicente	Sierra de Zapalinamé	Zapalinamé	BU Ejército Mexicano	El “Tulillo”	Villa Bilbao	Tomás Garrido	Sierra y Cañón de Jimulco
Kinosternidae (2 species)																			
<i>Kinosternon durangoense</i> *					+														
<i>Kinosternon flavescens</i>	+	+	+	+		+	+		+		+								
Testudinidae (2 species)																			
<i>Gopherus berlandieri</i>	+	+	+	+		+	+		+		+	+							
<i>Gopherus flavomarginatus</i> *					+														
Trionychidae (2 species)																			
<i>Apalone atra</i> **				+															
<i>Apalone spinifera</i>	+	+	+	+			+		+		+	+							
Totals	76	79	75	84	44	46	80	36	72	35	72	71	39	39	3	36	37	36	38

Of the 120 species included in Table 18, 26 (21.0%) are endemic species, including seven (5.8%) state endemics (*Gerrhonotus mccoysi*, *Crotaphytus antiquus*, *Uma exsul*, *Scincella kikaapoa*, *Terrapene coahuila*, *Trachemys taylori*, and *Apalone atra*). Ninety-one of the 120 species (75.8%) are non-endemics, and three (2.5%) are non-natives (all of the three that occur in the state). Naturally, it is not desirable to have the non-native species within protected areas, but, fortunately, only one of the three (*Hemidactylus turcicus*) is expected to be found in more than three of the areas (and it is expected in all of them).

Of the 23 species that are not expected to be found within the 19 existing protected areas, 12 are country endemics:

- Eleutherodactylus longipes*
- Rheohyla miotympanum*
- Ambystoma velasci*
- Aquiloerycea galeanae*
- Aquiloerycea scandens*
- Chiropterotriton priscus*
- Barisia imbricata*
- Phrynosoma orbiculare*
- Sceloporus cyanostictus*
- Sceloporus goldmani*
- Sceloporus minor*
- Scincella silvicola*

One of the 23 species (*Sceloporus gadsdeni*) is a state endemic and 10 are non-endemics (*Acris blanchardi*, *Smilisca baudinii*, *Holbrookia lacerata*, *Sceloporus*

marmoratus, *Lampropeltis gentilis*, *Tantilla cucullata*, *Trimorphodon wilkinsonii*, *Crotalus viridis*, *Terrapene ornata*, and *Kinosternon hirtipes*).

The principal herpetofaunal conservation goal for Coahuila, at this point, is to conduct herpetofaunal surveys in all currently-established conservation areas to determine which species are now actually represented. Based on this analysis, we predict that relatively few species are expected to be absent from all of these 19 areas, so a subsidiary goal is to ascertain whether this is the case and, if so, what other areas could be established to contain them.

Conclusions and Recommendations

Conclusions

- A. At the present time, the herpetofauna of Coahuila comprises 143 species, including 20 anurans, four salamanders, 106 squamates, and 13 turtles; three species are non-natives.
- B. The number of herpetofaunal species distributed among the 10 physiographic regions we recognize in Coahuila varies from 38 in the Laguna de Mayrán region to 91 in the Sierras y Llanuras Coahuilenses.
- C. The level of endemism of the herpetofauna of Coahuila is relatively low. Of the 143 species recorded from the state, 40 are endemic to Mexico, including nine limited to Coahuila. Thus, the percentage of endemism

Table 19. Summary of the distributional status of herpetofaunal species in protected areas in Coahuila, Mexico. Totals = total number of species recorded in all of the listed protected areas.

Protected Areas	Number of Species	Distributional Status			
		Non-endemic (NE)	Country Endemic (CE)	State Endemic (SE)	Non-native (NN)
Río Bravo del Norte	76	73	—	—	3
Maderas del Carmen	79	76	—	—	3
Ocampo	75	72	2	—	1
Cuatro Ciénegas	84	75	3	5	1
Mapimí	44	36	6	1	1
Los Novillos	46	45	—	—	1
Cuenca Alimentadora del Distrito Nacional de Riego 04 Don Martín	80	78	—	—	2
Distrito Nacional de Riego 026 Bajo Río San Juan.	36	30	5	—	1
Rancho Media Luna	72	71	—	—	1
Rancho La Puerta	35	31	3	—	1
Tierra Silvestre Cañón del Diablo	72	71	—	—	1
Sierra San Vicente	71	70	—	—	1
Sierra de Zapalinamé	39	30	8	—	1
Zapalinamé	39	30	8	—	1
Bosque Urbano Ejército Mexicano	3	2	—	—	1
El “Tulillo”	36	32	3	—	1
Villa Bilbao	37	35	—	1	1
Tomás Garrido	36	32	3	1	—
Sierra y Cañón de Jimulco	38	32	5	—	1
Totals	120	91	19	7	3

is 28.6%. The 40 endemic species amount to 4.9% of the 811 endemic species in Mexico.

D. The distributional status of the Coahuilan herpetofauna is as follows (in order of the size of the categories): non-endemics (100, 69.9%); country endemics (31, 21.7%); state endemics (nine, 6.3%); and non-natives (three, 2.1%).

E. The principal environmental threats in Coahuila are urban development, industrial pollution, deforestation for agricultural and ranching purposes, road effects, mining and energy projects, natural gas fracking, wind turbines, elimination due to cultural beliefs and practices, collecting and commercial trade, and forest fires.

F. The SEMARNAT, IUCN, and EVS systems were used to evaluate the conservation status of the herpetofauna of Coahuila. As demonstrated in previous MCS studies, the SEMARNAT system proved to be of little value, inasmuch as only 35.7% of the native herpetofauna has been evaluated to date. Of these 50 species, four are placed in the endangered category (P), 23 in the threatened category (A), and 23 in the special protection category (Pr).

G. The IUCN system was also applied to assess the native Coahuilan herpetofauna, and the results (by category

and proportion) are: CR (0 of 140 species; 0%); EN (7; 5.0%); VU (7; 5.0%); NT (8; 5.7%); LC (94; 67.1%); DD (1; 0.7%); and NE (23; 16.4%).

H. In addition, the EVS system was applied to the 140 native Coahuilan species. It placed them in the low, medium, and high vulnerability categories, and the values increased from low (33; 23.6%) to medium (55; 39.3%) and then slightly decreased in the high category (52; 37.1%).

I. The IUCN and EVS conservation status allocations ascertained that only 26.9% of the EVS high vulnerability species have been placed in two of the three IUCN threat categories (EN and VU; while no species are allocated to the CR category) and only 35.1% of the EVS low vulnerability species have been placed in the LC category. As such, the results of the application of these two systems do not correspond well to one another.

J. An analysis of the conservation status of the 118 species placed in the IUCN DD, NE, and LC categories demonstrates that many of them have been evaluated inappropriately compared to their respective EVS values. We opine that these species need to be reassessed to better reflect their prospects for survival.

K. The Relative Herpetofaunal Priority (RHP) measure



Fig. 23. *Forest Fires.* The scene after a forest fire in the vicinity of Arteaga, in the municipality of Arteaga. *Photo by Manuel Nevárez de los Reyes.*

was applied to establish the conservation significance of the ten regional herpetofaunas in Coahuila, which indicates that the most significant herpetofauna is that of the Gran Sierra Plegada, as it contains the greatest number of country endemics and the second greatest number of high vulnerability species. The other nine physiographic regions are arranged in decreasing order of significance on the basis of their number of endemic species, as follows: Sierra Transversales; Sierras y Llanuras Coahuilenses; Bolsón de Mapimí; Pliegues Saltillo Parras; Laguna de Mayrán and Sierra de la Paila; Llanuras y Sierras Volcánicas, Serranías del Burro, and Llanuras de Coahuila y Nuevo León. On the basis of their numbers of high vulnerability species, the ranking is as follows: Sierras y Llanuras Coahuilenses; Gran Sierra Plegada; Sierras Transversales; Pliegues Saltillo Parras; Bolsón de Mapimí; Llanuras y Sierras Volcánicas and Serranía del Burro; Laguna de Mayrán, Sierra de la Paila, and Llanuras de Coahuila y Nuevo León.

L. Nineteen protected areas have been established in Coahuila; eight federal reserves, four federal/private reserves, three state reserves, three state/private reserves, and one municipal reserve. The representation of these 19 areas among the ten physiographic areas is weighted in favor of the Sierras y Llanuras Coahuilenses (eight areas), which ranked 3rd in endemic species and 1st in high vulnerability species. The Gran Sierra Plegada is the next best represented (in four areas). Unfortunately, all of the 19 protected areas for which information is available are occupied by landowners. In addition, few areas have the

benefit of herpetofaunal surveys or management plans.

M. Our analyses predict that 120 of 143 total species are expected to be found in the 19 protected areas (83.9%). These 120 species include 91 non-endemics, 19 country endemics, seven state endemics, and three non-natives. The non-native species should not be included in the protected areas system.

N. Future conservation efforts should be directed toward conducting thorough herpetofaunal surveys in all components of the protected areas system, as well as determining what additional areas might be required to provide protection for all of Coahuila's herpetofaunal species.

Recommendations

A. Our principal interest in writing this paper has been to assess the conservation status of the 140 native species presently recorded from the state of Coahuila, and to suggest what steps need to be taken to protect all of these species over the long term. We have undertaken this assessment using the EVS methodology, as we have in the previous entries in the Mexican Conservation Series, which demonstrated that 33 species are allocated to the low vulnerability category, 55 to the medium vulnerability category, and 52 to the high vulnerability category. We also employed the Relative Herpetofaunal Priority methodology to determine which of the physiographic regions in the



No. 21. *Crotalus lepidus* (Kennicott, 1861). The Rock Rattlesnake is distributed “in the United States in southeastern Arizona, southern New Mexico, and western Texas” and “in Mexico... in Sonora, Chihuahua, Durango, Sinaloa, Nayarit, Jalisco, Zacatecas, Aguascalientes, Coahuila, Nuevo León, and San Luis Potosí” (Prival and Porter 2016: 444). This individual came from Jimenez, in the municipality of Jimenez. Wilson et al. (2013a) evaluated its EVS as 12, placing it in the upper portion of the medium vulnerability category. Its conservation status is considered as Least Concern by IUCN and as a species of special protection (Pr) by SEMARNAT. *Photo by Michael S. Price.*



No. 22. *Crotalus pricei* Van Denburgh, 1895. The Twin-spotted Rattlesnake ranges “from southeastern Arizona in the United States (Chiricahua, Huachuca, Pinaleno, Dos Cabezas, and Santa Rita mountains) southward in Mexico through the Sierra Madre Occidental to northeastern Sonora, western Chihuahua, and Durango, and in the Sierra Madre Oriental of southeastern Coahuila, southern Nuevo Leon, southwestern Tamaulipas, and north-central San Luis Potosi, and in Aguascalientes” (Hammerson et al. 2007). This individual was found at Monterreal, in the municipality of Arteaga. Wilson et al. (2013a) assessed its EVS as 14, placing it at the lower limit of the high vulnerability category. Its conservation status is judged as Least Concern by IUCN and as a species of special protection (Pr) by SEMARNAT. *Photo by Elí García Padilla.*



No. 23. *Sistrurus tergeminus* (Say, 1823). The Western Massasauga occurs in the USA (Texas, Oklahoma) and Mexico (central and northeastern Coahuila, southern Nuevo León; possibly in Tamaulipas, northern Chihuahua, and northeastern Sonora). This individual came from 6 km south of La Piedra Parada, in the municipality of Guerrero. The EVS value of this rattlesnake is 13. Its conservation status has not been assessed by IUCN and it is not listed by SEMARNAT. *Photo by Manuel Nevarez de los Reyes.*



No. 24. *Terrapene coahuila* Schmidt and Owens, 1944. The Coahuila Box Turtle is a Mexican endemic species restricted to “the Cuatro Ciénegas Bolson of Coahuila” (Lemos-Espinal et al. 2015: 122). This individual was located at Cuatrociénegas in the municipality of Cuatrociénegas de Carranza. Wilson et al. (2013a) calculated its EVS as 19, placing it in the upper portion of the high vulnerability category. Its conservation status is determined as Endangered by IUCN and as threatened (A) by SEMARNAT. *Photo by Michael S. Price.*



No. 25. *Trachemys taylori* (Legler, 1960). The Cuatro Ciénegas Slider is an endemic Mexican species restricted in distribution to the Cuatro Ciénegas Basin (Lemos-Espinal et al. 2015). This individual came from Cuatrociénegas in the municipality of Cuatrociénegas de Carranza. Wilson et al. (2013a) determined its EVS as 19, placing it in the upper portion of the high vulnerability category. Its conservation status is calculated as Endangered by IUCN, but this species is not listed by SEMARNAT. *Photo by Michael S. Price.*

state support the most significant herpetofaunas based on the relative numbers of country endemics and high vulnerability species. Three such areas were identified: the Gran Sierra Plegada, the Sierras Transversales, and the Sierras y Llanuras Coahuilenses. Fortunately, these three regions support the greatest numbers of protected areas among the 19 that are currently established, four, four, and eight, respectively. The herpetofaunal content of these protected areas, however, is very poorly known; as a result, the major conservation goal with respect to the herpetofauna of Coahuila is to carefully document the species inhabiting the protected areas of the state in order to test the predictions made here about their content and to draw up adequate management plans for their perpetual protection.

B. Thus, it will only be after the species inhabiting the existing protected areas have been identified that additional conservation goals can be addressed. These goals include (1) determining what other protected areas might need to be established to protect the remainder of the herpetofauna not found within the existing areas, (2) monitoring of the health of the populations of species within the protected areas, and (3) assessing the well-being of the ecosystems on which these species depend.

C. It is imperative that this work advance as rapidly as possible, especially given that efforts to protect

the Coahuilan herpetofauna lag behind those of the other Mexican states examined thus far in the Mexican Conservation Series.

“We have come a very long way through the barbaric period in which we still live, and now I believe we have learned enough to adopt a transcendent moral precept concerning the rest of life. It is simple and easy to say: Do no further harm to the biosphere.”—E.O. Wilson (2016)

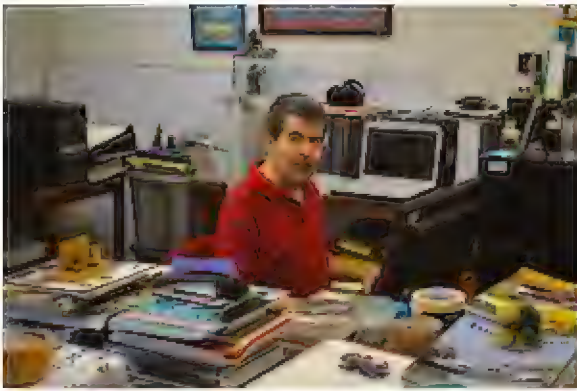
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Literature Cited

Alvarado-Díaz J, Suazo-Ortuño I, Wilson LD, Medina-Aguilar O. 2013. Patterns of physiographic distribution and conservation status of the

- herpetofauna of Michoacán, Mexico. *Amphibian & Reptile Conservation* 7: 128–170 (e71).
- Anderson CG, Greenbaum E. 2012. Phylogeography of northern populations of the Black-tailed Rattlesnake (*Crotalus molossus* Baird and Girard, 1853), with the revalidation of *C. ornatus* Hallowell, 1854. *Herpetological Monographs* 26: 19–57.
- Baeza-Tarín F, Hernández T, Giovanetto L, Espinosa Treviño A, Lazcano D, Graham SP. 2018a. Geographic distribution. *Lampropeltis gentilis* (Western Milksnake). *Herpetological Review* 49: 505.
- Baeza-Tarín F, Hernández T, Giovanetto L, Espinosa Treviño A, Lazcano D, Graham SP. 2018b. Geographic distribution. *Tantilla cucullata* (Trans-Pecos Black-headed Snake). *Herpetological Review* 49: 507–508.
- Baeza-Tarín F, Hernández T, Giovanetto L, Espinosa Treviño A, Lazcano D, Graham SP. 2018c. Geographic distribution. *Trimorphodon wilkinsonii* (Texas Lyresnake). *Herpetological Review* 49: 508.
- Banda-Leal J, Lazcano D, Barriga-Vallejo C, Nevárez-de los Reyes M. 2018. New records of *Gerrhonotus parvus* Knight & Scudday, 1985 (Squamata, Anguidae) in the state of Coahuila, México. *Check List* 14: 1–6.
- Cerdá-Ardura A, Soberón-Mobarak F, McGaugh SE, Vogt RC. 2008. *Apalone spinifera atra* (Webb and Legler 1960) – Black Spiny Softshell Turtle, Cuatrociénegas softshell, tortuga concha blanda, tortuga negra de Cuatrociénegas. Pp. 021.1–021.4 In: *Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group*. Chelonian Research Monographs No. 5. Editors, Rhodin AGJ, Pritchard PCH, van Dijk PP, Saumure RA, Buhlmann KA, Iverson JB. Chelonian Research Foundation, Lunenburg, Massachusetts, USA. Various pagination.
- Chávez Cisneros JA, Lazcano D, Salinas Camarena MA. 2010. *Cophosaurus texanus* (Greater Earless Lizard). Mortality. *Herpetological Review* 41: 75.
- Cruz-Elizalde R, Ramírez-Bautista A, Johnson JD, Moreno CE. 2014. Community structure of reptiles from the southern portion of the Chihuahuan Desert Region, Mexico. *North-Western Journal of Zoology* 10: 173–182.
- Cruz-Sáenz D, Muñoz-Nolasco FJ, Mata-Silva V, Johnson JD, García-Padilla E, Wilson LD. 2017. The herpetofauna of Jalisco, Mexico: composition, distribution, and conservation status. *Mesoamerican Herpetology* 4: 22–118.
- DeSantis DL, García-Padilla E, Wilson LD, Mata-Silva V. 2018. Conservation of herpetofauna in disturbed habitats: perspectives from short-term surveys in the Sierra Madre del Sur, Oaxaca, Mexico. Pp. 165–192 In: *Ecología y Conservación de Fauna en Ambientes Antropizados*. Editors, Ramírez-Bautista A, Pineda-López R. CONACYT-REFAMA, Querétaro, Mexico. 203 p.
- Díaz-Cárdenas B, Ruiz-Sanchez E, Castro-Felix P, Castañeda-Gaytán G, Ruiz-Santana S, Gadsden H. 2017. Species delimitation of the Blue-spotted Spiny Lizard within a multi-locus, multispecies coalescent framework, results in the recognition of a new *Sceloporus* species. *Molecular Phylogenetics and Evolution* 111: 185–195.
- Dodd CK Jr. 2013. *Frogs of the United States and Canada*. 2 vols. Johns Hopkins University Press, Baltimore, Maryland, USA. 1,025 p.
- Frost DR. 2018. *Amphibian Species of the World: an Online Reference*. Version 6.0. American Museum of Natural History, New York, New York, USA. Available: <http://www.research.amnh.org/herpetology/amphibia/index.html> [Accessed: 8 March 2018].
- García-Vázquez UO, Contreras-Arquieta A, Trujano-Ortega M, Nieto-Montes de Oca A. 2018. A new species of *Gerrhonotus* (Squamata: Anguidae) from the Cuatro Ciénegas Basin, Coahuila, Mexico. *Herpetologica* 74: 269–278.
- González-Sánchez VH, Johnson JD, García-Padilla E, Mata-Silva V, DeSantis DL, Wilson LD. 2017. The herpetofauna of the Mexican Yucatan Peninsula: composition, distribution, and conservation status. *Mesoamerican Herpetology* 4: 264–380.
- Johnson JD, Mata-Silva V, García-Padilla E, Wilson LD. 2015a. The herpetofauna of Chiapas, Mexico: composition, distribution, and conservation. *Mesoamerican Herpetology* 2: 271–329.
- Johnson JD, Mata-Silva V, Wilson LD. 2015b. A conservation reassessment of the Central American herpetofauna based on the EVS measure. *Amphibian & Reptile Conservation* 9 [General Section]: 1–94 (e100).
- Johnson JD, Wilson LD, Mata-Silva V, García-Padilla E, DeSantis DL. 2017. The endemic herpetofauna of Mexico: organisms of global significance in severe peril. *Mesoamerican Herpetology* 4: 543–620.
- Lazcano D, García de la Peña C, Castañeda G. 2006. *Gerrhonotus liocephalus* (Texas Alligator Lizard). Mortality. *Herpetological Review* 37: 222.
- Lazcano D, Jacobo Galván RD, García de la Peña C, Castañeda GG. 2006. *Phrynosoma cornutum* (Texas Horned Lizard). Mortality. *Herpetological Review* 37: 91.
- Lazcano D, Bailón-Cuellar E, Ruiz-Ayma G, Mercado-Hernández R, Navarro-Velázquez B, Wilson LD, Powell GL, Russell AP. 2017. Texas Horned Lizards (*Phrynosoma cornutum*) as prey of Swainson's Hawk (*Buteo swainsoni*) nest sites at La Reserva de la Biosfera de Janos, Chihuahua, Mexico. *Mesoamerican Herpetology* 4: 885–900.
- Leaché AD. 2009. Species tree discordance traces to phylogeographic clade boundaries in North American fence lizards (*Sceloporus*). *Systematic Biology* 58: 547–559.

- Leaché AD, Mulcahy DG. 2007. Phylogeny, divergence times and species limits of spiny lizards (*Sceloporus magister* species group) in western North American deserts and Baja California. *Molecular Ecology* 16: 5,216–5,233.
- Lemos-Espinal JA, Smith HM. 2007. *Anfibios y Reptiles del Estado de Coahuila, México/Amphibians and Reptiles of the State of Coahuila, Mexico*. CONABIO, México, Distrito Federal, Mexico. 550 p.
- Lemos-Espinal JA. (Editor) 2015. *Amphibians and Reptiles of the US-Mexico Border States/Anfibios y Reptiles de los Estados de la Frontera México-Estados Unidos*. Texas A&M University Press, College Station, Texas, USA. 614 p.
- Lemos-Espinal JA, Smith HM, Dixon JR, Cruz A. 2015. *Anfibios y Reptiles de Sonora, Chihuahua y Coahuila, México/Amphibians and Reptiles of Sonora, Chihuahua, and Coahuila, Mexico*. CONABIO, México, Distrito Federal, México. 348 p.
- Lemos-Espinal JA, Smith GR. 2016. Amphibians and reptiles of the state of Coahuila, Mexico, with comparison with adjoining states. *ZooKeys* 593: 117–137.
- Martínez-Méndez N, Méndez-de la Cruz FR. 2007. Molecular phylogeny of the *Sceloporus torquatus* species-group (Squamata: Phrynosomatidae). *Zootaxa* 1609: 53–68.
- Mata-Silva V, Johnson JD, Wilson LD, García Padilla E. 2015. The herpetofauna of Oaxaca, Mexico: composition, distribution, and conservation. *Mesoamerican Herpetology* 2: 5–62.
- Mata-Silva V, DeSantis DL, García-Padilla E, Johnson JD, Wilson LD. 2019. The endemic herpetofauna of Central America: a casualty of anthropocentrism. *Amphibian & Reptile Conservation* 13(1) [General Section]: 1–64 (e168).
- Mendoza-Quijano F, Flores-Villela O, Sites JW Jr. 1998. Genetic variation, species status, and phylogenetic relationships in Rose-Bellied Lizards (*Variabilis* Group) of the genus *Sceloporus* (Squamata: Phrynosomatidae). *Copeia* 1998: 354–366.
- Nevárez de los Reyes M, Lazcano D, García-Padilla E, Mata-Silva V, Johnson JD, Wilson LD. 2016. The herpetofauna of Nuevo León, Mexico: composition, distribution, and conservation. *Mesoamerican Herpetology* 3: 557–638.
- Pyron RA, Burbrink FT. 2009. Systematics of the Common Kingsnake (*Lampropeltis getula*; Serpentes: Colubridae) and the burden of heritage in taxonomy. *Zootaxa* 2241: 22–32.
- Ruane S, Bryson RW Jr, Pyron RA, Burbrink FT. 2014. Coalescent species delimitation in milksnakes (genus *Lampropeltis*) and impacts on phylogenetic comparative analyses. *Systematic Biology* 63: 231–250.
- Schulte JA II, Macey JR, Papenfuss TJ. 2006. A genetic perspective on the geographic association of taxa among arid North American lizards of the *Sceloporus magister* complex (Squamata: Iguanidae: Phrynosomatinae). *Molecular Phylogenetics and Evolution* 39: 873–880.
- Serb JM, Phillips CA, Iverson JB. 2001. Molecular phylogeny and biogeography of *Kinosternon flavescens* based on complete mitochondrial control region sequences. *Molecular Phylogenetics and Evolution* 18: 149–162.
- Servicio Geológico Mexicano Secretaría de Energía. 2017. *Panorama Minero del Estado de Coahuila*. Available: <http://www.sgm.gob.mx/pdfs/COAHUILA.pdf> [Accessed: 21 July 2019]
- SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales). 2010. *Norma Oficial Mexicana Nom-059-SEMARNAT-2010, Protección Ambiental-Especies Nativas de México de Flora y Fauna Silvestres-Categorías de Riesgo y Especificaciones para su Inclusión, Exclusión o Cambio-Lista de Especies en Riesgo. Diario Oficial de la Federación, 30 de Diciembre de 2010*. SEMARNAT, México, Distrito Federal, Mexico. Available: <http://www.dof.gob.mx/normasOficiales/4254/semarnat/semarnat.htm> [Accessed: 21 July 2019].
- Smith HM, Smith RB. 1979. *Synopsis of the Herpetofauna of Mexico, Vol. VI: Guide to Mexican Turtles, Bibliographic Addendum III*. John Johnson, North Bennington, Vermont, USA. 1,044 p.
- Terán-Juárez SA, García-Padilla E, Mata-Silva V, Johnson JD, Wilson LD. 2016. The herpetofauna of Tamaulipas, Mexico: composition, distribution, and conservation. *Mesoamerican Herpetology* 3: 42–113.
- Wilson EO. 2016. *Half Earth: Our Planet's Fight for Life*. Liveright Publishing Corporation, New York, New York, USA. 259 p.
- Wilson LD, McCranie JR. 2004. The conservation status of the herpetofauna of Honduras. *Amphibian & Reptile Conservation* 3: 6–33 (e12).
- Wilson LD, Mata-Silva V, Johnson JD. 2013a. A conservation reassessment of the reptiles of Mexico based on the EVS measure. Contribution to Special Mexico Issue. *Amphibian & Reptile Conservation* 7(1): 1–47 (e61).
- Wilson LD, Johnson JD, Mata-Silva V. 2013b. A conservation reassessment of the amphibians of Mexico based on the EVS measure. Contribution to Special Mexico Issue. *Amphibian & Reptile Conservation* 7(1): 97–127 (e69).
- Woolrich-Piña GA, Ramírez-Silva JP, Loc-Barragán J, Ponce Campos P, Mata-Silva V, Johnson JD, García Padilla E, Wilson LD. 2016. The herpetofauna of Nayarit, Mexico: composition, distribution, and conservation. *Mesoamerican Herpetology* 3: 375–448.
- Woolrich-Piña GA, García-Padilla E, DeSantis DL, Johnson JD, Mata-Silva V, Wilson LD. 2017. The herpetofauna of Puebla, Mexico: composition, distribution, and conservation status. *Mesoamerican Herpetology* 4: 790–884.



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Manuel Nevárez-de los Reyes is a biologist who graduated from the Universidad Autónoma de Nuevo León (UANL), Facultad de Ciencias Biológicas in San Nicolás de los Garza, México. Manuel's initial interest was in the study of amphibians and reptiles, but his professional life led him to investigate other areas, such as environmental impacts and the study of cacti. From 1997 to 2007, he served as head of Environmental Protection in the Residencia Regional de Construcción Noreste of the Federal Electricity Commission. Manuel has been involved with numerous workshops and conferences, and has authored both popular science and peer-reviewed articles on herpetology and cacti. Among his accomplishments, he discovered and co-authored the original description of a new genus and species of cactus, *Digitostigma caput-medusae*. The following year he created "Proyecto Digitostigma," a nursery dedicated to the commercial propagation of various cacti, which contributes to their knowledge and conservation. Manuel recently obtained his Ph.D. in Wildlife Management and Sustainable Development at the UANL, with a thesis entitled "Ecological distribution of the herpetofauna of the Sierra de Gomas in northern Nuevo León," under a grant from the National Council of Science and Technology. He is now part of the herpetological group that has documented the many herpetological activities in northeastern México (Coahuila, Nuevo León, and Estado de México).



Elí García-Padilla is a herpetologist primarily focused on the ecology and natural history of the Mexican herpetofauna. His research efforts have centered on the Mexican states of Baja California, Tamaulipas, Chiapas, and Oaxaca. His first experience in the field was studying the ecology of the insular endemic populations of the rattlesnakes *Crotalus catalinensis*, *C. muertensis* (*C. pyrrhus*), and *C. tortugensis* (*C. atrox*) in the Gulf of California. Elí's Bachelor's thesis was on the ecology of *C. muertensis* (*C. pyrrhus*) on Isla El Muerto, Baja California, Mexico. To date, he has authored or co-authored over 100 contributions to science. Elí is currently a formal Curator of Amphibians and Reptiles from Mexico in the electronic platform "Naturalista" of the Comisión Nacional para el Uso y Conocimiento de la Biodiversidad (CONABIO-inaturalist; <http://www.naturalista.mx>). One of his main passions is environmental education, and for several years he has worked on various projects that include the use of photography and audiovisual media as a powerful tool for reaching large audiences and promoting the knowledge, protection, and conservation of Mexican biodiversity. Elí's interests include wildlife and conservation photography, and his art has been published in several scientific, artistic, and educational books, magazines, and websites. Presently, he is collaborating on an evaluation of the jaguar (*Panthera onca*) as an umbrella species for the conservation of the herpetofauna of Nuclear Central America.

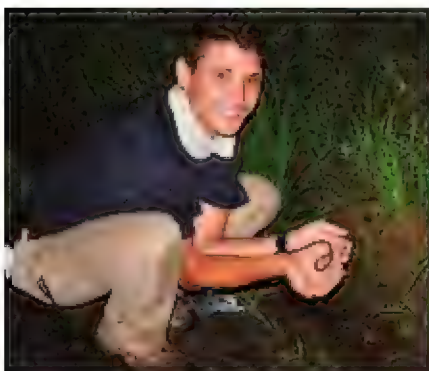


Jerry D. Johnson is Professor of Biological Sciences at The University of Texas at El Paso (UTEP), and has been investigating the systematics, ecology, and conservation of the herpetofauna of Middle America since 1970, especially that of southern Mexico. Jerry is also the Director of UTEP's 40,000-acre Indio Mountains Research Station in the Chihuahuan Desert of Trans-Pecos, Texas. He has authored or co-authored over 120 peer-reviewed papers, and was co-editor or contributor to several major Mesoamerican herpetology books: *Conservation of Mesoamerican Amphibians and Reptiles*, *Mesoamerican Herpetology: Systematics, Zoogeography, and Conservation*, and *Middle American Herpetology: A Bibliographic Checklist*. One species, *Tantilla johnsoni*, was named in his honor. Presently, Jerry is an Associate Editor and Co-chair of the Taxonomic Board of the *Mesoamerican Herpetology* website.

The herpetofauna of Coahuila, Mexico



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Dominic L. DeSantis is currently a Ph.D. candidate and National Science Foundation Graduate Research Fellow at the University of Texas at El Paso. He received his Bachelor's degree at Texas State University, where he also completed multiple research projects on the antipredator behavior of the critically endangered Barton Springs Salamander (*Eurycea sosorum*). Dominic's ongoing dissertation research integrates multiple field monitoring technologies to study snake movement and behavioral ecology. Dominic accompanied Vicente Mata-Silva, Eli García-Padilla, and Larry David Wilson on survey and collecting trips to Oaxaca in 2015, 2016, and 2017, and he is a co-author on numerous natural history publications produced from those visits.



Larry David Wilson is a herpetologist with lengthy experience in Mesoamerica. He was born in Taylorville, Illinois, USA, and received his university education at the University of Illinois at Champaign-Urbana (B.S. degree) and at Louisiana State University in Baton Rouge (M.S. and Ph.D. degrees). He has authored or co-authored over 410 peer-reviewed papers and books on herpetology. Larry was the senior editor or author of several books, including *Conservation of Mesoamerican Amphibians and Reptiles*, *The Snakes of Honduras*, *Middle American Herpetology*, *The Amphibians of Honduras*, *Amphibians & Reptiles of the Bay Islands and Cayos Cochinos, Honduras*, *The Amphibians and Reptiles of the Honduran Mosquitia*, and *Guide to the Amphibians & Reptiles of Cusuco National Park, Honduras*. To date, he has authored or co-authored the descriptions of 72 currently recognized herpetofaunal species, and seven species have been named in his honor, including the anuran *Craugastor lauraster*, the lizard *Norops wilsoni*, and the snakes *Oxybelis wilsoni*, *Myriopholis wilsoni*, and *Cerrophidion wilsoni*. Larry previously served as an Associate Editor and is presently Co-chair of the Taxonomic Board for the journal *Mesoamerican Herpetology*.



Unpublished population data of *Dendrobates azureus* Hoogmoed 1969 obtained in 1968 and 1970, and its historical and current taxonomic status

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Abstract.—During a herpetological inventory in the Sipaliwini area in southern Suriname in 1968, and again during a second expedition to the area in 1970, anecdotal population data on *Dendrobates azureus* Hoogmoed, 1969 were obtained. As of now, some 50 years later, these data have not been published, yet they may be useful for the evaluation of the status of this taxon at the present time and the evolution of its populations over the period since 1968. Visits to the Sipaliwini savanna to observe or collect this taxon over the past 50 years have been few and far between. An overview of the population data available in the publications about these visits is provided.

Keywords. *Dendrobates tinctorius*, isolated populations, Sipaliwini savanna, Suriname, conservation, threats.

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Introduction

During a herpetological inventory of the Sipaliwini area in 1968 Hoogmoed (1969a,b, 1971a,b, 1972) discovered a blue Dendrobatid frog in forest islands in the Sipaliwini savanna, near the Vier Gebroeders Mountain close to the Suriname-Brazilian border, that he named *Dendrobates azureus*. In the description (Hoogmoed 1969b), a number of ecological and behavioral data were provided, but data that give an impression about the abundance of the population were not presented, because they were not collected in a systematic way, but rather anecdotally. The author paid a second visit to the Sipaliwini area and the habitat of *D. azureus* in 1970 (Hoogmoed 1971a,b, 1972) and more data were obtained on the abundance of the frog in the type locality (forest island on West slope Vier Gebroeders Mountain) and some other forest islands nearby, and about forest island occupation by this taxon. These population data are available in the author's field books (archived in the former Rijksmuseum van Natuurlijke Historie, now Naturalis Biodiversity Center in Leiden [RMNH], in the Netherlands) and in his private diary notes, and they can provide basic data about the population status of the taxon at the time of its discovery. This taxon is considered vulnerable [VU: D2] (Stuart et al. 2006), and as a member of the genus *Dendrobates* it is on CITES Appendix II, so in Suriname it officially cannot be traded (Hoogmoed 2013). However, since it still has been subject to illegal capture and export of specimens,

it seems timely to publish the basic population data of this taxon, even though they were collected anecdotally.

Polder (1974), based on his observations of the taxon in captivity, expressed some doubt on the specific status of *D. azureus*, but this had no direct consequences as Silverstone (1975) considered it a species. Wollenberg et al. (2006), followed by Wollenberg (2007), on the basis of morphological data and genetic analysis, synonymized *D. azureus* with *D. tinctorius* (Cuvier, 1797). This assessment was supported by Noonan and Gaucher (2006), who found that specimens of *D. azureus* (from the type locality or near to it, see below, Noonan, pers. comm.) shared the same haplotype with two “nearby” (from the continuous rainforest, see below, Noonan, pers. comm.) populations of *D. tinctorius* and concluded that this signified the two taxa were identical. Grant et al. (2006) still treated *D. azureus* as a species but on the basis of molecular results were inclined to follow the synonymy suggested by Wollenberg et al. (2006). Gaucher and McCulloch (2010) and Frost (2018) accepted the synonymization and considered *D. azureus* a synonym of *D. tinctorius*. Ouboter and Jairam (2012) considered *D. azureus* a subspecies of *D. tinctorius*, without providing arguments, but this was not accepted by Frost (2018), who treated *D. tinctorius* as monotypic.

However, Avila-Pires et al. (2010) and Hoogmoed (2013) did not accept the synonymization by Wollenberg et al. (2006) and pointed out that this publication suffered several shortcomings. Here I add to those critiques the

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use of tissue samples of captive bred material of dubious origin (although stated to be from the type locality) and the fact that among dendrobatid breeders *D. azureus* has been confused with, and interbred with, a blue morph of *D. tinctorius* that occurs in southern Guyana, northwestern Pará in Brazil (south to Porto Trombetas) and possibly in extreme southwestern Suriname (in a contested area between Suriname and Guyana). See Avila-Pires et al (2010: Fig. 23) and Lötters et al. (2007: Fig. 707) for color pictures of this blue *D. tinctorius* morph. Hoogmoed (1969, 2013: Fig. 4), Eisenberg (2004), and Lötters et al. (2007: Fig. 708 [as *D. tinctorius*]) provide pictures of *D. azureus*. Noonan and Gaucher (2006) mention specimens of *D. tinctorius* and *D. azureus* from slightly different localities (Table 1) and their samples of *D. azureus* are from the area of Vier Gebroeders Mountain (see Noonan, pers. comm., below as well). In Wollenberg et al. (2008) it seems that the blue morph of *D. tinctorius* has incorrectly been considered as *D. azureus* (see Fig. 4 Haplotype 2, the second figure from above and the lower figure; no *D. azureus* can be found in this figure). Unfortunately, only the fancy names of hobbyists have been used for the “Sipaliwini” material and no vouchers have been indicated.

In order to remove any doubt regarding which population I am discussing, and avoid upsetting the present accepted nomenclature, below I use the name *Dendrobates “azureus”* in the sense of the population of *D. tinctorius* described in 1969 as *D. azureus* and only known from isolated forest islands in the Sipaliwini savanna in southern Suriname.

History of (the herpetological) exploration of the Sipaliwini Savanna and inventories of *D. “azureus”* populations

1935–1938: Border expedition (van Lynden 1939) to establish the border between Suriname and Brazil (= watershed). Although Van Lynden stayed on the Sipaliwini savanna for an extended period (4 October 1935 to 10 March 1936), and made general observations about animals (mostly mammals and birds), he did not mention “blue frogs,” so we may conclude he did not observe them, or that he did not deem them worthy of mentioning. His camp III (on the western base of Vier Gebroeders Mountain, from where he wrote his diary on 12 October 1935) actually was close to the later type locality of *D. “azureus,”* but it was probably in the savanna itself, not in the forest island. The map published by van Lynden (1939) unfortunately does not show the location of the forest islands.

1961: During Operation Grasshopper, the Sipaliwini airstrip was constructed on a small savanna about 3.5 km west of the western border of the Sipaliwini savanna. Apparently, the forest islands in the savanna were not visited.

1968: Hoogmoed (1969a,b) discovered populations of *D. “azureus”* in four forest islands in the middle of the Sipaliwini savanna near the Vier Gebroeders Mountain



Fig. 1. *Dendrobates “azureus”* (= *tinctorius*).

when making a herpetological inventory of the area in 1968 (22 August to 7 October), concentrating on the area between Sipaliwini airstrip and Vier Gebroeders Mountain. He collected a total of 37 specimens and two tadpoles of *D. “azureus”* (Table 1), which were all preserved and form the type material of the description of *D. azureus* Hoogmoed, 1969. The location and shape of the forest islands as shown in Hoogmoed (1969b: Fig. 3) were taken from a topographical map (Centraal Bureau Luchtkartering, Paramaribo, Suriname) of the area based on aerial photographs.

1968–1969: After Hoogmoed’s departure from the area, the Sipaliwini expedition (Hoogmoed 1969a) continued working in the Sipaliwini savanna and the participants (botanists and a geologist) moved N of the Vier Gebroeders area to the Morro Grande Mountain area. Hoogmoed (1969b: Fig. 3) marked four northern forest islands as possible localities for *D. “azureus”* because the botanist J.P. Schulz reported having seen “blue frogs” there. Considering later observations (see Gagliardo 2004 a,b; Fouquet et al. 2015), these might well have been *D. tinctorius* with a yellow semicircular mark on the snout.

1970: Between 13 January and 13 February Hoogmoed again visited the Sipaliwini savanna, this time concentrating on the part south of the Vier Gebroeders Mountain and the Brazilian border. During this trip one specimen of *D. “azureus”* was collected and preserved, and 10 additional specimens were collected at the type locality and transported alive to the Netherlands, where they were bred in captivity by Polder (1973a–c, 1974).

1981: According to Wevers (2007), a “group of Dutchmen” brought live *D. “azureus”* from the Sipaliwini savanna (most likely an illegal operation), making no mention of the names of participants, numbers of frogs brought back, or from which forest island(s).

1988: An illegal import of *D. “azureus”* (apparently three specimens) was confiscated in the Netherlands and transferred to Blijdorp Zoo, Rotterdam (Wevers 2007).

1996: Cover (1996, 1997) reported on an expedition in June 1996 (sponsored by the National Aquarium in Baltimore, USA) to attempt a population survey of *D.*

Table 1. Detailed data for specimens of *D. “azureus”* collected/observed by M.S. Hoogmoed in 1968 and 1970. Number of specimens accounts for both collected and observed specimens, numbers between brackets in the first column refer to the numbers of forest islands as used in the text. The asterisk (*) indicates that 10 specimens were observed in about 10 minutes.

Forest island (see text)	Date	Number of specimens	Time spent in field	Person-minutes spent in field	Number person- minutes per specimen
1968					
1	10 Sep	1 (RMNH 13838)	18:15 h	-	-
1	11 Sep	11 (RMNH 13837)	10:30–12:30 h	120	10.9
1	13 Sep	15	15:00–15:30 h	30	2
1	30 Sep	1 (+ 2 tadpoles) (RMNH 13842)	10:15 h	-	-
1	1 Oct	10 (RMNH 13843)	11:00–11:30 h	30	3
1	Mean or total	38		180	4.7
2	19 Sep	5 (RMNH 13839)	10:00–11:30 h	90	18
3	20 Sep	0	-	120	-
3	23 Sep	10 (6 coll, RMNH 13840)	10:00–11:00 h	60	10*
3	Mean or total	10		180	18
4	27 Sep	3 (RMNH 13841)	10:30–12:00 h	90	30
1970					
1	6 Feb	1 (RMNH 42487)	13:15 h	-	-
1	7 Feb	25	08:00–12:00 h (2 persons)	480	19.2
Total 1968 + 1970					
		82 (38 preserved, 10 live for ex situ breeding colony)	11 days	920	10.1

“*azureus*.” Three staff members of the NAIB and three field workers of Conservation International Suriname participated. Fifty-four adults and two juveniles were observed during a limited number of days. They surveyed six forest islands and found specimens in three of them “two on the slopes of Vier Gebroeders and one in a valley floor forest just north of the mountains” (Cover 1997; Eiben 2005). Most likely Cover referred to forest island nos. 1 and 4 on the slopes of Vier Gebroeders Mountain and to forest island no. 2 north of that mountain (see below). No material was collected, and the position of the other three forest islands was not mentioned.

1997: Gagliardo (2004a,b) reported on a new expedition (14 August–19 September 1997) by Cover and three other zoo curators in order to collect specimens to establish a breeding population in the USA. “Nearly 60 specimens” and an unknown number of tadpoles were observed in two forest islands that were not the type locality. Furthermore, three pairs of *D. tinctorius* (with a yellow semi-circular mark on the snout) were collected

(and finally exported) in a forest island northeast of the Sipaliwini airstrip. One day before departure from the savanna permission was obtained to collect and export 20 specimens of *D. “azureus,”* so they had to be collected at a rush and were meant to establish an ex-situ breeding population (Eiben 2005). No mention was made of which forest island(s) these specimens were collected from or how much time it took to collect them.

2003: B.P. Noonan (pers. comm.; Eiben 2005) visited the area of the Vier Gebroeders Mountain from 23–26 May 2003. He flew in using Mamija airstrip (= “Myers’ airstrip” in Hoogmoed (1969) and in the present text, Wapaisana Anotato on Google Earth) on the border of Suriname and Brazil, SE of the Vier Gebroeders Mountain, and he left via Sipaliwini airstrip, W of the savanna. He was not allowed to collect specimens of *D. “azureus,”* but was allowed to make toe clips from 10 specimens for molecular studies (Noonan and Gaucher 2006). He found three specimens on 24 May, four specimens on 25 May, and another three on 26 May. No

data on time spent finding specimens are available, but Noonan writes: “...my experience was that neither of these populations was terribly dense. While I did not keep detailed notes on abundance, I am comfortable saying that I did not observe more than one individual per hour of searching (on average).” Noonan also collected tissue from three *Dendrobates* specimens found in the continuous rainforest NE of the airstrip Sipaliwini, about 100 m from the savanna edge, that were identified as *D. tinctorius* (Noonan and Gaucher 2006).

2007: According to Wevers (2007) several frog fanciers visited the Sipaliwini savanna and “observed respectively 9, 15 [probably Wevers himself] and 20 specimens (mostly the same specimens).” Wevers (2007) visited the Sipaliwini savanna for five days in February and during those days observed 15 specimens and four larvae. He at least visited the type locality on the western slope of the Vier Gebroeders Mountain and the forest island on the northeastern slope from where he reported juvenile specimens. He reported that his guide who lived on Mamija airstrip (= “Myers’ airstrip”) on the frontier of Suriname and Brazil, never had seen more than 25 *D. “azureus”* in one day. Based on his own observations (five days and a limited number of forest islands visited) and information from his Indian guides, he estimated the size of the total wild population to be between 1,000 and 1,500 specimens, but this does not seem to be a very reliable figure.

2014: Fouquet et al. (2015) visited the Sipaliwini area between 15 and 28 April, but did not visit the forest islands where *D. “azureus”* occurs. They reported *D. tinctorius* (with a yellow semi-circular mark on the snout) from a mountain 10 km N of Sipaliwini airstrip in the area of continuous rainforest.

By no means is this overview intended to be an exhaustive listing of all visits to the *D. “azureus”* habitat or nearby areas. It is known that Suriname scientists with a license to study the frogs and personnel of the Forestry Service flew into Sipaliwini airstrip, but were not allowed to travel from there to the Vier Gebroeders Mountain and they were confined to the airstrip. Some scientist may have paid unregistered (and unpublished) visits to the area. Illegal collectors (animal dealers) and terrarium keepers apparently have visited the area at least several times, but because of the nature of these trips, they have not been documented publicly. It also is possible that native and Brazilian Indian collectors have provided animal dealers with specimens that may have left Suriname directly or via Brazil. No numerical data are available, but Suriname animal dealers exporting reptiles and amphibians to the USA and Europe have long-standing commercial contacts (since the early 1970’s) with the Indians of the villages of Alalapadu and Kwamalasemutu.

Material and Methods

During the 1968 herpetological inventory of the Sipaliwini savanna in southern Suriname (Hoogmoed 1969a,b), five forest islands near the Vier Gebroeders Mountain were

searched. In 1970, Hoogmoed surveyed five forest islands in the southern part of the savanna. During the fieldwork in the area around Vier Gebroeders Mountain no formal population surveys were made, but notes were kept about how many specimens were observed/collected during the time spent along transects in the forest islands. Frogs were observed/collected while traversing forest islands following creek beds, either downhill or ascending the creek, generally searching an area of five m at each side of the stream. The time period during which frogs were collected was noted, and based on this the abundance was expressed in specimens per person-minutes. In 1968 observations/collections were made by one person, and in 1970 by two people. All specimens were either simply observed, or collected by hand. Specimens collected were killed with MS222, fixed and preserved in 70% ethanol (thus, no formaldehyde was used and the type specimens could still be used for DNA analysis). Live specimens were transported in plastic bags with leaf litter, and termites were provided as food.

Data on specimens collected in 1968 were provided by Hoogmoed (1969b) in general terms. The coordinates of forest islands where *D. “azureus”* was found were calculated in 1968 on the basis of a topographic map of the area, but they now can be provided more precisely, based on localization with Google maps. Only slight differences can be noticed.

In 1968, the area of the Sipaliwini savanna where *D. “azureus”* was obtained was visited between 11 September and 1 October, a total of 21 days. During this period, several forest islands on and near the Vier Gebroeders Mountain (as well as the intervening savanna area) were searched for herpetofauna. Coordinates for the center of the forest islands are given as in Hoogmoed (1969b) and corrected according to Google Earth 2018, datum W84. Forest islands inhabited by *D. “azureus”* are indicated with asterisks (*).

1. *Forest island W flank Vier Gebroeders Mountain (Base Bivouac), type locality of *D. azureus*, 2°N, 55°58’W (corrected to 2°00’21.24”N, 55°58’10.85”W)
2. *Forest island (J-shaped [the eastern narrow extension is not rainforest but gallery forest of *Mauritia* palms]) 1.5 km NE of Vier Gebroeders Mountain, 2°01’N, 55°57.30”W (corrected to 2°00’59.30”N, 55°57’26.03”W)
3. *Forest-island 2 km (note this distance differs from that in the description of *D. azureus*) N of Vier Gebroeders Mountain, long and narrow, directed W–E, 2°01’N, 55°58’W (corrected to 2°01’25.78”N, 55°57’34.22”W)
4. *Forest island on NE slope Vier Gebroeders Mountain, 2°N, 55°57’30”W (corrected to: 2°00’24.92”N, 55°57’22.03”W)
5. Forest island (small) on N slope Vier Gebroeders Mountain (Google Earth 2°00’52.49”N, 55°58’04.71”W)

In 1970, the Sipaliwini savanna was visited again (13 January–13 February), this time mostly in a part further south from the area visited in 1968, with a stay of only

two days in forest island no. 1 (see above). During this period the following forest islands, that turned out not to be inhabited by *D. “azureus,”* were searched (coordinates based on Google Earth 2018):

6. Small forest island on northernmost part of Lange Dijk, 2°00'09.36"N, 55°55'58.78"W, Suriname, 27 January 1970
7. Small forest island on ridge of Lange Dijk, 1°59'28.01"N, 55°55'15.81"W, Brazil, 27 January 1970
8. Elongate forest island on SW slope Lange Dijk, 1°59'18.74"N, 55°55'20.73"W, Brazil, 27 January 1970
9. Westernmost small forest island of two, E of Myers' farm, about 8 km WSW of Vier Gebroeders Mountain W flank, 1°59'09.10"N, 56°02'35.11"W, Suriname, 4 February 1970
10. Easternmost (630 m E of and twice as large as no. 9) forest island, E. of Myers' farm, about 8 km WSW of Vier Gebroeders Mountain W flank, 1°59'11.78"N, 56°02'13.63"W, Suriname, 4 February 1970.

Results

Forest Islands

The shapes and sizes of eight forest islands in 1968 were based on a topographical map and aerial photographs that formed the basis for Figure 3 in Hoogmoed (1969b). These were all compared with Google Earth images of 31 December 1969 and 17 November 2004 (the most recent freely available large-scale images on the Internet), and all the forest islands still exist and no notable changes in shape or size were observed. Cover (1997) noted that concern had been expressed that the anthropogenic fires which ravage the savanna yearly might damage the forest islands. In 1970, Hoogmoed observed that savanna fire had destroyed the narrow band of forest between the 1968 Vier Gebroeders Bivouac (type locality) and the savanna on the SW edge of the campground, but also that the forest island interior, probably because it is rather moist, had not suffered any damage. *D. “azureus”* was still regularly present in the former, open camp ground. Gagliardi (2004a,b) stated that the fires did enter the forest islands, but he did not mention the extent of damage. Wevers (2007) wrote that fire had gnawed at the edges of the forest islands and expressed fear that in an El Niño year fire might reach the interior of the forest islands and thus threaten their integrity. Cover (1997), however, reported that the fires apparently did not damage the forest islands, but thought that they might be the reason that the forest islands did not expand into the savanna. These last observations are confirmed by Hoogmoed's 1970 observations (Hoogmoed 1972) and by the Google Earth images of 2004. The *Map for Environment* (2018) shows that there has been only limited tree loss in the Sipaliwini savanna between 2000 and 2014, although tree loss near Sipaliwini airstrip has been significant. The forest islands themselves do not show any noticeable changes.

The most recent Google Earth images (2004) show that in the Brazilian part of the savanna (Paru savanna) south and east of the Sipaliwini savanna there are six large forest islands that would be worth investigating for the presence of *D. “azureus.”* However, as this area is a Brazilian Indian Territory, conducting biological research there is very difficult, because of the need for special permits and its remoteness. Just south of the Paru savanna in Brazil is another, isolated, more or less oval savanna with a large, elongate forest island in the middle (160 km SSW of Sipaliwini airstrip). This forest island was inventoried by Avila-Pires et al. (2010: ESEC Grão Pará Centro) and they did not find any *Dendrobates* species there.

Population Data

11 September–1 October 1968. For this period of 21 days spent in Vier Gebroeders Bivouac, general herpetological collecting was conducted in the savanna and forest islands on and near Vier Gebroeders Mountain. Only some parts of the days were spent in forest islands searching for *D. “azureus.”*

Apart from the four forest islands where specimens were observed and collected, one small forest island on the N slope of Vier Gebroeders Mountain was searched for *D. “azureus,”* but no specimens were found. The four northernmost forest islands in Hoogmoed's (1969) map were not visited during this time. Data on time spent observing/collecting *D. “azureus”* and population density are summarized in Table 1.

Between 1968 and 1970. The population density of *D. “azureus”* in forest island no. 1 on the W slope of the Vier Gebroeders Mountain seems to have diminished considerably (remembering that 23 specimens and two tadpoles were removed in 1968, which might have had a negative influence on the population), viz. one specimen per 4.7 person-minutes in 1968 (one observer only), versus one specimen per 19.2 person-minutes in 1970 (two observers).

In 1970 an additional 11 specimens were removed from this same forest island, one for the RMNH collection, and ten live specimens to establish an ex-situ breeding colony in the Netherlands.

It should be mentioned that specimens were not evenly distributed throughout the forest islands. They might be absent in certain stretches and be numerous in other parts (generally near creeks and/or in areas with large boulders).

No comparative data for the other forest-islands are available for the period 1968–1970. Cover (1996, 1997) does not provide data in a comparable way, but he apparently collected data in three of the forest islands mentioned by Hoogmoed (1969), but unfortunately the locations of these have not been published. Noonan (pers. comm. 2017) reported that he did not see more than one specimen per hour. Already in 1968, the population den-

sity in the other forest islands seemed to be less (one specimen per 18–30 person-minutes) than in the largest forest island (no. 1) on the W slope of Vier Gebroeders Mountain (one specimen per 4.7 person-minutes). This could be related to the size of the forest islands, but this is just an impression that is not based on firm facts. Also, we have to take into account that more time was spent in forest island no 1, because it was the location of the camp (both in 1968 and 1970).

Collections in 1968 were made during the second part of September, during the dry season, and those in 1970 were made in early February, during the beginning of the wet season—when rainfall is about twice that in the dry season, and about half that of May and June, the wettest months (see Hoogmoed 1969).

Conclusions

At the time of the discovery of *D. “azureus”* in 1968 it was clear that not all forest islands inhabited by this taxon had populations of the same density. Since 1968, although several expeditions have visited the distribution area of *D. “azureus”* in the Sipaliwini savanna, no data on population densities have been published that could be directly compared with those presented here. However, the anecdotal data available (see the **History of ... inventories of *D. “azureus”* populations** section above) give the strong impression that the numbers of *D. “azureus”* in its restricted habitat have considerably diminished since 1968. This impression should be confirmed by systematic population studies that might serve in situ and ex situ management programs for this unique population of brilliant blue poison frogs. At the moment we do not even have an idea about the size of the population in the wild, but it might run only into the hundreds. Eiben (2005) and Stuart et al. (2008) described the successful ex situ breeding program in the National Aquarium in Baltimore (Maryland, USA) based on 20 specimens collected in 1997 (see above) and some additional exchanged specimens. This program should be continued and fortified with the help of the Suriname authorities and several nature conservation interest groups, such as WWF, The Nature Conservancy, and Conservation International, that are already active in Suriname.

Although the habitat of this taxon is completely within a Suriname Nature Reserve, the area is easily accessible from Brazil and the border is not patrolled. Illegal visits by collectors cannot be ignored, and should be taken into account when making an in situ management plan. Stuart et al. (2008: 228) optimistically assumed that interest in wild collected specimens would diminish with successful breeding in captivity, but this is a naïve assumption (e.g., IUCN 2015).

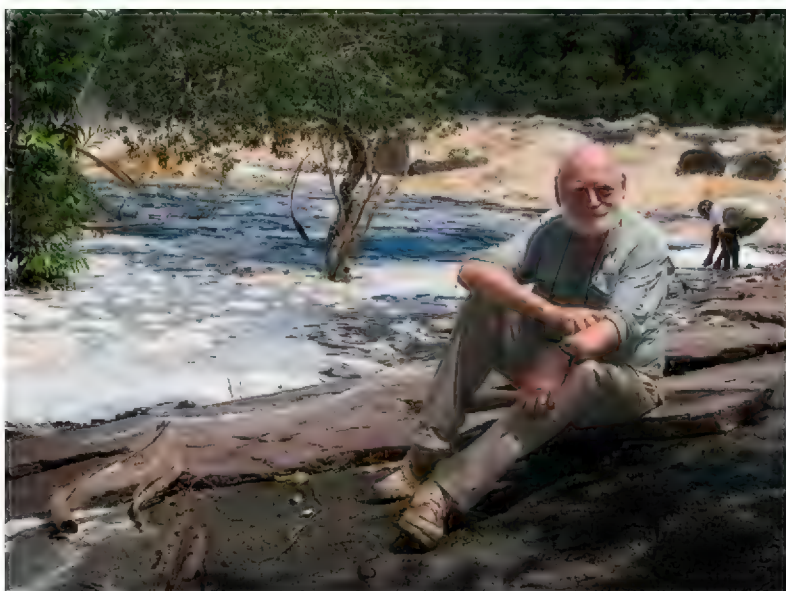
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Literature Cited

- Avila-Pires TCS, Hoogmoed MS, Rocha WA. 2010. Notes on the vertebrates of northern Pará, Brazil: a forgotten part of the Guianan Region, I. Herpetofauna. *Boletim do Museu Paraense Emílio Goeldi. Ciências Naturais* 5(1): 13–112.
- Cover JF. 1996. Jungle Journal: in search of the Okopipi. *Watermarks. Member’s Quarterly of the National Aquarium in Baltimore* 1996(Fall): 4–10.
- Cover JF. 1997. A pilot field study of the Blue Poison-Dart frog (*Dendrobates azureus*) in Suriname. *American Zoo and Aquarium Association. Regional Conference Cleveland, Ohio, Proceeding* 1997: 492–497.
- Eiben B. 2005. Blue Dart-Poison Frog (*Dendrobates azureus*) [1996–present]. In: Zippel K. Zoos play a vital role in amphibian conservation. Available: <http://www.amphibiaweb.org/declines/zoo/index.html> [Accessed: 20 July 2017].
- Eisenberg T. 2004. *Der Blaue Pfeilgiftfrosch Dendrobates azureus*. Terrarienbibliothek Art für Art, Natur und Tier Verlag GmbH, Münster, Germany. 63 p.
- Frost DR. 2018. Amphibian Species of the World: an Online Reference. Version 6.0. American Museum of Natural History, New York, New York, USA. Available: <http://research.amnh.org/herpetology/amphibia/index.html> [Accessed: 20 February 2018].
- Gagliardo R. 2004a. Sipaliwini overleven op zoek naar *Dendrobates azureus*. *DN Magazine* 16(March): 24–27.
- Gagliardo R. 2004b. Auf der Suche nach dem Blauen Pfeilgiftfrosch. Sipaliwini – Überleben auf der Suche nach *Dendrobates azureus*. *Faszination Pfeilgiftfrösche Journal* 3: 6–15.
- Gaucher P, MacCulloch R. 2010. *Dendrobates tinctorius*. The IUCN Red List of Threatened Species 2010: e.T55204A11265402. Available: <http://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T55204A11265402.en> [Accessed: 20 February 2018].
- Grant T, Frost DR, Caldwell TP, Gagliardo R, Haddad CFB, Kok PJR, Means DB, Noonan BP, Schargel WE, Wheeler WC. 2006. Phylogenetic systematics of Dart-Poison Frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). *Bulletin of the American Museum of Natural History* 299: 1–262.
- Hoogmoed MS. 1969a. Notes on the herpetofauna of Suriname I. – Itinerary of a herpetological collecting trip in Suriname in 1968. *Zoologische Mededelingen* 44(4): 47–73.
- Hoogmoed MS. 1969b. Notes on the herpetofauna

- of Suriname III. – A new species of *Dendrobates* (Amphibia: Salientia: Dendrobatidae) from Suriname. *Zoologische Mededelingen* 44(9): 133–141.
- Hoogmoed MS. 1971a. *Dendrobates*, een kleurrijk genus. *Het Aquarium* 41(8): 182–189.
- Hoogmoed MS. 1971b. *Dendrobates*, eine farbenreiche Gattung. *Die Aquarien- und Terrarien-Zeitschrift (DATZ)* 24(1): 1–7.
- Hoogmoed MS. 1972. Frösche, die ihre Kaulquappen hükepack tragen. *Aquarien Magazine* 1972(7): 288–293.
- Hoogmoed MS. 2013. Status and conservation of amphibians in Suriname. Pp. 231–279 In: Heatwole H, Barrio-Amorós CL, Wilkinson JW. *Status and Decline of Amphibians: Western Hemisphere Part 3, Venezuela, Guyana, Suriname, and French Guiana*. Amphibian Biology 9. Surrey Beatty and Sons, Baulkham Hills, NSW, Australia. VIII, 197–298 p.
- IUCN. 2016. Fact sheet: *Dendrobates tinctorius* (Amphibia: Anura: Dendrobatidae). *CITES AC 28 Information Documents* 35: 1–8.
- Lötters S, Jungfer KH, Henkel FW, Schmidt W. 2007. *Poison Frogs: Biology, Species and Captive Care*. Edition Chimaira, Frankfurt am Main, Germany. 668 p.
- Lynden AJH van. 1939. Op zoek naar de Zuidgrens. De grensbepaling tusschen Suriname en Brazilië, 1935–1938. *Tijdschrift van het Koninklijk Nederlandsch Aardrijkskundig Genootschap* 56(6): 1–90.
- Map for Environment. 2018. Tree losses in Cerrado Biome. Available: <https://mapforenvironment.org/map/view/522/#3.72/2.28/-48.98> [Accessed: 13 March 2018].
- Noonan BP, Gaucher P. 2006. Refugial isolation and secondary contact in the dyeing poison frog *Dendrobates tinctorius*. *Molecular Ecology* 15: 4,425–4,435.
- Ouboter PE, Jairam R. 2012. *Amphibians of Suriname*. E.J. Brill, Leiden, Netherlands. 376 p.
- Polder WN. 1973a. Over verzorging en voortplanting in gevangenschap van *Dendrobates azureus* en enkele andere Dendrobatidae. *Het Aquarium* 44(1): 16–22.
- Polder WN. 1973b. Over verzorging en voortplanting in gevangenschap van *Dendrobates azureus* en enkele andere Dendrobatidae (2). *Het Aquarium* 44(7): 186–191.
- Polder WN. 1973c. Over verzorging en voortplanting in gevangenschap van *Dendrobates azureus* en enkele andere Dendrobatidae (III). *Het Aquarium* 44(12): 324–330.
- Polder WN. 1974. Over verzorging en voortplanting in gevangenschap van *Dendrobates azureus* en enkele andere Dendrobatidae IV. *Het Aquarium* 45(5): 122–128.
- Silverstone PA. 1975. A revision of the poison-arrow frogs of the genus *Dendrobates* Wagler. *Natural History Museum Los Angeles County Science Bulletin* 21: 1–55.
- Stuart SN, Hoffmann M, Chanson JS, Cox NA, Berridge RJ, Ramani P, Young BE. 2006. *Threatened Amphibians of the World*. Lynx Editions, Barcelona, Spain; IUCN, Gland, Switzerland; Conservation International, Arlington, Virginia, USA; NatureServe, Costa Rica. 758 p.
- Wevers E. 2007. In het biotoop van de Okopipi. *Onder het Palmblad* 10: 14–23.
- Wollenberg KC, Veith M, Noonan BP, Lötters S. 2006. Polymorphism versus species richness—Systematics of large *Dendrobates* from the Eastern Guiana Shield (Amphibia: Dendrobatidae). *Copeia* 2006(4): 623–629.
- Wollenberg C. 2007. *Dendrobates tinctorius* — Polymorphismus oder Artenreichtum? *Aquaristik Fachmagazin und Aquarium Heute* (194) 39(2): 18–21.
- Wollenberg KC, Lötters S, Mora-Ferrer C, Veith M. 2008. Disentangling composite color patterns in a poison frog species. *Biological Journal of the Linnean Society* 93: 433–444.



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Vulnerability of Northern Pine Snakes (*Pituophis melanoleucus* Daudin, 1803) during fall den ingress in New Jersey, USA

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Abstract.—The management of threatened and endangered species often falls to various state agencies which may have different and conflicting goals. The Pine Barrens of New Jersey are managed for different objectives, including fire management, tree cutting, recreational activities (hiking, hunting, off-road-vehicle use), wildlife protection, and conservation. Managing competing claims requires ecological information on critical issues and vulnerabilities for determining the impacts of each claim. The Northern Pine Snake (*Pituophis melanoleucus*), an iconic Pine Barrens species that is threatened in New Jersey, is normally dispersed during spring and summer, but the snakes converge in the fall to communal hibernacula, where they spend the winter and leave in the spring. Here, the activity of Northern Pine Snakes near hibernacula in the fall is described to examine their vulnerability to various competing claims, such as fire or off-road vehicle use. Two hypotheses are tested: (i) that snakes enter the hibernaculum once (and stay), and (ii) that the total period of ingress for all Northern Pine Snakes is limited to just a few weeks in the fall. Activity of PIT-tagged snakes at hibernacula entrances was monitored with a passive, continuously-recording AVID Tracker and temperatures were monitored with a continuously recording thermometer placed at the soil surface. The behavior of marked snakes (18 in 2017, 25 in 2018), indicated that the period of activity around the hibernaculum entrance was: 1) longer than expected (i.e., over two months), 2) involved multiple ingress and egress of individual snakes, and 3) sometimes involved movement between two or among multiple nearby hibernacula. Northern Pine Snakes generally did not move in or out of hibernacula when temperatures were below 9° C. Daytime high and nighttime low temperatures greatly influenced movement. Although the daily high and low temperatures when snakes moved were correlated ($r = 0.54$ in 2017; 0.51 in 2018, $P < 0.0001$), the daily high and low temperatures were more highly correlated ($r = 0.71$ in 2017; 0.79 in 2018), indicating factors other than temperature influence snake activities. Most snakes entered and exited between 1000 and 1800 h, although some moved as late as 0030 h. These data can inform science-based decisions about when to allow tree cutting, fire management, and off-road vehicle races (e.g., increased human activity). Most snakes are concentrated around hibernacula (but not necessarily near the entrances) from early October until early December (or the end of December for two hatchlings). Therefore, a significant proportion of snakes are vulnerable to disturbances that could impact their population viability. Vulnerabilities are discussed in terms of competing claims and conservation.

Keywords. Behavior, competing claims, hibernation, reptiles, Serpentes, Squamata, wildlife management

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Introduction

Managers are often required to make environmental resource decisions with incomplete knowledge, with little time, and often under conditions of competing claims for resources and associated habitat. Human alteration of natural lands is a key driver of global biodiversity loss (Pimm and Raven 2000; Wilcove et al. 2000). Claims for land can come from those who want to either use the

resource itself (e.g., hunting, fishing, wildlife collecting), use important components of the habitat (e.g., logging), or improve the habitat for people (e.g., trail or road building, fire suppression). Indeed, managers of different resources (e.g., timber, wildlife) are often in conflict, asserting competing claims for the same resource or habitat. These need to be carefully considered and resolved in a manner that reduces the risks to the habitat and wildlife, while enhancing human benefits. For example,

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managers of forests must balance cutting (or harvesting) trees against re-forestation, the adverse effects of cutting trees against the benefits of cutting them, and the relative importance of the different benefits and costs (McLeod and Gates 1998; Todd and Andrews 2008). Similarly, fire suppression has some benefits (e.g., reduced potential for fire damage to nearby communities and industries), as well as costs if it does not occur (e.g., allowing dry debris to build-up, creating the potential for a very hot canopy fire when it does occur). Each option (e.g., logging, fire suppression) has both benefits and costs to the plants and animals living in the forests (Roth and Franklin 2018; Steen et al. 2010). In addition, most animals face one or more challenges to their survival, including predators, competitors, poachers, and resource users, as well as threats to their habitat from development, wildfire, or resource use. What may be good for one animal group may not be good for another. For example, creating gaps in forests may be good for deer management and for snakes requiring open nesting areas, but is not good for interior-nesting forest birds (Blouin-Demers and Weatherhead 2001; Gerald et al. 2006; MWPARC 2009). The complex situations forest managers face can only be resolved by examining the ecological and societal benefits and costs of different options.

Setting priorities for conservation is a challenging and necessary effort (Pimm et al. 2001). The biodiversity crisis facing the Earth suggests that the conservation needs of threatened and endangered species should be considered first when managing habitats and ecosystems (Wilson et al. 2009; Gaiarsa et al. 2015). Economic, societal, and political issues also play important roles in conservation decisions (Polasky 2008; Wilson et al. 2011). However, it is equally important to understand the roles of species vulnerability (IUCN 2009), habitat loss (Wilson 1992; Pimm et al. 1995; Gibbons et al. 2000), habitat fragmentation and patch size (Forman and Godron 1986; Hilton-Taylor 2000; Kjooss and Litvaitis 2001; Sander-son et al. 2002), restricted range or habitats (Segura et al. 2007; Cardillo et al. 2008), human disturbances (Parent and Weatherhead 2000), human infrastructures (e.g., roads, Andrews et al. 2008), and environmental stochasticity (Tanentzap et al. 2012), among others (Gaiarsa et al. 2015). Non-random distributions in time and space are important aspects of vulnerability for animals, particularly those that are slow moving or temperature-dependent (Croak et al. 2013), including ectothermic vertebrates (Kapfer et al. 2010). Understanding temporal and spatial use of core areas is critical for conservation and management (Semlitsch and Bodie 2003). All of these factors become more important for understanding life histories and conservation when they are considered within a framework of human-related activities and impacts (e.g., fire management, logging, development; Kapfer et al. 2010).

One of the iconic species of the New Jersey Pine Barrens is the Northern Pine Snake (*Pituophis melanoleucus* Daudin, 1803), a top-level predator that can reach 2 m in

length. The Northern Pine Snake is listed as a threatened species in New Jersey and as threatened or endangered in other parts of its range in the southern United States (Burger et al. 2017, 2018). New Jersey appears to have the most stable population of this species (thus, a more global responsibility for its conservation, Golden et al. 2009; Burger and Zappalorti 2016; Burger et al. 2017). This paper examines the behavior of individually PIT-tagged Northern Pine Snakes during their fall ingress into hibernation sites (hibernacula or winter dens) in relation to conservation of the species with respect to forest management (e.g., logging, fire suppression) and other human activities (e.g., poaching, off-road vehicle [ORV] races, and traffic). There are competing claims for the habitat (e.g., snake use and ORV use), for habitat management (e.g., fire management and deer management), and for the snakes themselves (e.g., population stability and poaching, Burger and Zappalorti 2016). The activities of snakes in the fall were monitored around several hibernacula using new passive PIT-tag recording devices located at the hibernaculum entrances. This technical application is described with the intent of illustrating its use for other such studies. The overall goal was to test the null hypothesis (H_0) that Northern Pine Snakes return to their hibernacula and enter only once, remaining there for the duration of the winter hibernation period. If H_0 is rejected, this could indicate increased vulnerability in time and space for a snake that is already threatened in New Jersey.

Hibernation behavior in this species has been studied previously in terms of hibernaculum site selection, use and fidelity, structure of hibernacula, and the defensive behavior of snakes disturbed during hibernation (Burger et al. 1988, 2000; Burger and Zappalorti 2016, 2017). Hibernaculum sites of other species of pine snakes (*P. ruthveni* and *P. melanoleucus lodingi*) were studied in Mississippi where the former used burrows of small mammals, and the latter used decayed pine stumps and roots for hibernation (Rudolph et al. 2007). Dispersal rates around hibernation sites were also examined in Gopher Snakes (*Pituophis catenifer deserticola*) in British Columbia (Williams et al. 2012). Some studies on hibernation in other snake species include hibernation site selection (Harvey and Weatherhead 2006), body temperatures while hibernating (Costanzo 1986; Hein and Guyer 2009), cold tolerance (Joy and Crews 1987), factors affecting spring emergence (Todd et al. 2009), spring emergence patterns (Hirth et al. 1969; Gregory 1974; Shine et al. 2006), and gene flow among snakes in different hibernacula (Clark et al. 2008; Anderson 2010). However, without individually marked snakes, and the availability of the recent remote recording ability of the Avid Power Tracker VIII, it was previously impossible to determine the activity of individual snakes near hibernation sites, the final entry temperatures for fall ingress, or the final dates of entry into hibernacula, each of which were incorporated into the present study.

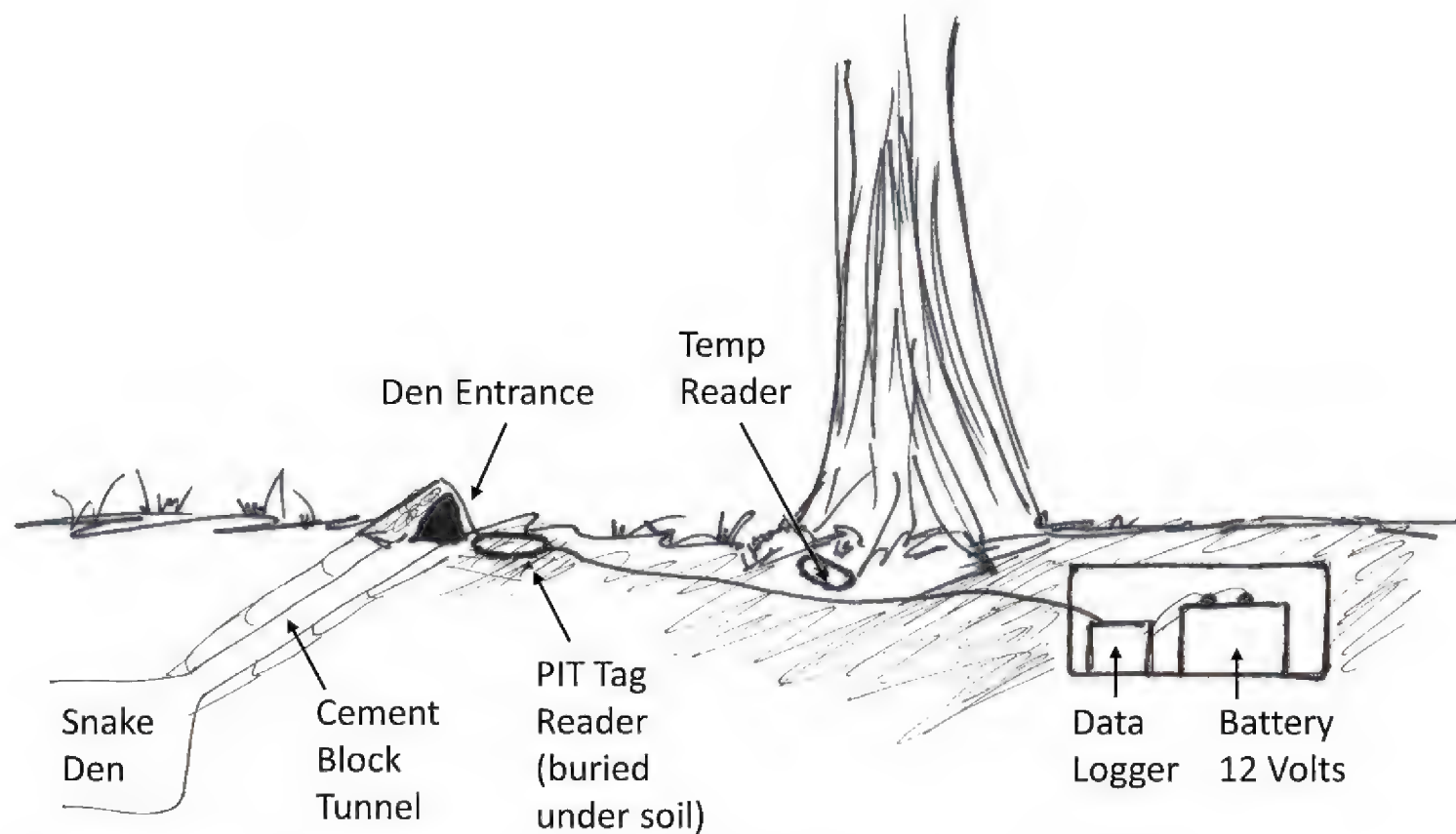


Fig. 1. Schematic of deployment of the Tracker device at the entrance of a hibernaculum.

Methods

Northern Pine Snakes have been studied in the Pine Barrens of New Jersey for over 35 years, including marking each snake with an individual PIT tag (Burger and Zappalorti 2011). They are large constrictors that reach the northern limit of their range in southern New Jersey. The New Jersey population is separated from other populations by several hundred km (Golden et al. 2009; Burger and Zappalorti 2011, 2016). They dig their own nest burrows, and dig or modify hibernacula (Burger and Zappalorti 2011). They hibernate communally, along with other snake species.

Northern Pine Snake studies have examined breeding and hibernation biology, risks and threats Northern Pine Snakes face, habitat selection, movement and home ranges, and contaminant exposure. While movement has been studied throughout the year with the use of radio-tracking, these previous studies did not provide detailed movement of the community of snakes using hibernacula (Burger and Zappalorti 2011). During this period Northern Pine Snakes were studied in Burlington, Cumberland, and Ocean counties, however, the exact locations of the studies were not disclosed because of the very high risk of poaching of Northern Pine Snakes (Burger et al. 2017, 2018).

The present study used passive continuous recording devices on snakes as they left and entered the hibernacula. In 2017 one hibernaculum was monitored to test the feasibility of using this tracking method, and in 2018, five hibernacula were monitored (four units monitored snakes for the entire year in Bass River State Forest, and one additional site was only monitored in the fall in Berkeley Township, Ocean County, known as “Davenport Den”). Any tagged snake passing by, entering, or leaving one of these monitored hibernacula was recorded. Data were generally down-loaded every 2–3 weeks throughout the

year. A recording device was placed at the entrance of each hibernaculum, and buried so it was not visible (Fig. 1). The device used was the AVID Power TracKer VIII, a multi-mode reader with memory for PIT tags, made by AVID Identification Systems, Inc., in Norco, California. A TracKer unit was placed at each hibernaculum entrance and covered with a 1 cm layer of sand to prevent vandalism. The unit has a 6-inch coil reader with leads that can be up to 4 m long and lead to a device that can record and store up to 2,500 events, recording the PIT Tag, the time of day, and the date for each event. The power source was a 12-volt marine battery. The recorder and battery were placed in a plastic box, covered with a board for stability (and to prevent collapse if someone stepped on it by accident), buried beneath 10 cm of soil, and covered with leaves and twigs for camouflage (Fig. 1). None of the equipment was visible on the surface to prevent injury of the snakes, theft, or vandalism. The technology requires that snakes are fitted with PIT tags. Although the recorders were operating all year, this report only examines snake activity from 15 October to 31 December 2017 (at one hibernaculum) and from 1 October to 31 December 2018 (at five hibernacula).

The soil surface temperatures were recorded continuously, all year, near one of the hibernaculum entrances at Bass River State Forest using an Elitech RC-5USB Temperature Data Logger. The device was placed in a plastic bag and covered with a 1 cm layer of sand and moss to disguise its location. In previous studies, this small recorder has worked for well over a year on its original battery.

This study was only possible because: 1) Many Northern Pine Snakes use the same traditional hibernation and nesting sites (Burger and Zappalorti 2011; Burger et al. 2012; Zappalorti et al. 2014), 2) Gravid female Northern Pine Snake use the same hibernating and nesting sites (Burger and Zappalorti 1992, 2015), 3) Hatchlings can

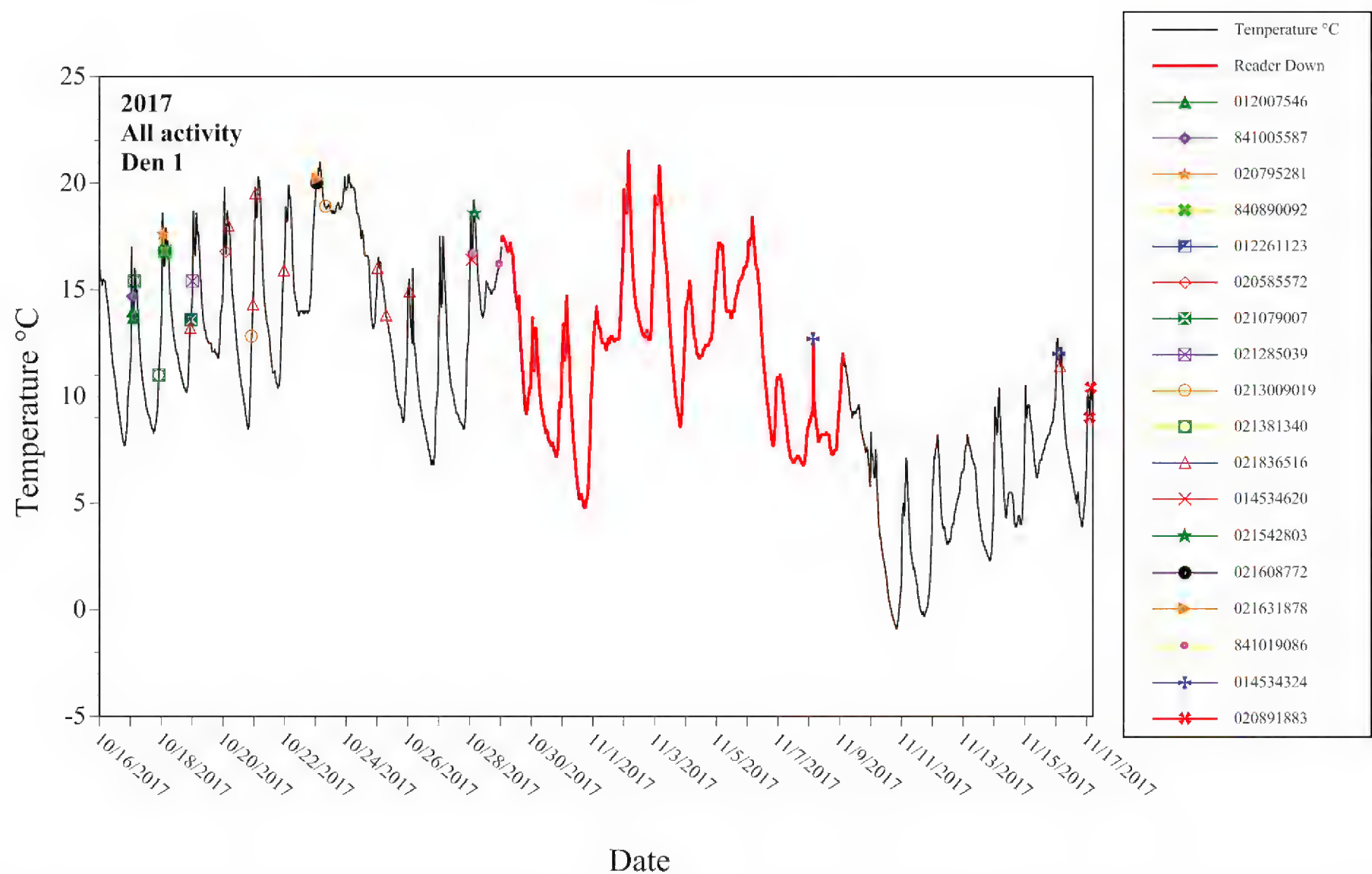


Fig. 2. All activity of snakes at den 1 (Bass River State Forest) in 2017 as a function of date and soil surface temperature. The colored markers indicated by 9-digit numbers in the legend represent individually tagged snakes. Data for snakes during the period from 29 October to 11 November (red line) were not recorded because the maximum number of data points the receiver could store was reached.

be easily found at nesting areas, fitted with PIT tags, and then followed at the hibernacula, and 4) Therefore, most of the individual snakes using a given hibernation site are PIT-tagged. Further, the sexes and ages of all snakes were known because they had been followed since they were hatchlings or two-to-three years old. The hibernacula in this study had been studied for over 30 years, and were well-known to the snakes and the researchers (Burger and Zappalorti 2011; Burger et al. 2012). For each snake, the first reading hit of the season was assumed to be its initial entry.

Analyses included calculating frequencies, percentages, means, and standard deviations for various behavior parameters of the Northern Pine Snake around hibernation sites in the fall. Data were analyzed using standard SAS software (Statistical Analysis Systems, Cary, North Carolina, USA), including Kruskal-Wallis One-Way Analysis of Variance (ANOVA), with 95% confidence intervals. Kendall tau was used to determine correlations among ambient soil surface temperatures and the temperatures at which snakes exhibited activity. The best models for explaining variations in snake activity as a function of temperature and date were developed using SAS (ProcGLM) procedures. Variables included were den, year, age, sex, date (only for the temperature model), and maximum and minimum daily sand surface temperatures.

Results

Date and temperature: The two primary factors that might account for entry of Northern Pine Snakes into hibernacula in the fall are date and ambient temperature. The factors entering the best model ($F = 34$, $P < 0.0001$, $r^2 = 76$) for Julian date of activity (e.g., entering/leaving) were maximum daytime sand temperature ($P < 0.001$), den number ($P < 0.04$), and perhaps age ($P < 0.08$). The factors entering the best model ($F = 122$, $P < 0.0001$, $r^2 = 90$) explaining variation in the temperature of snake activity (i.e., soil surface temperature when a snake entered, left, or passed by the hibernaculum) were maximum daytime sand temperature ($P < 0.001$), minimum nighttime temperature ($P < 0.0001$), and sex ($P < 0.04$). These factors are explored in greater detail below.

Seasonal activity patterns: While there was virtually no activity around the hibernacula in August or September, by early October snakes returned to the vicinity of the dens and began passing by, entering, and leaving hibernacula. In 2017, the equipment was only deployed in mid-October (when activity was expected to begin) at Bass River State Forest, but many snakes were already active around den 1 (Fig. 2). In the first year the reader was initially placed 0.3 m down the tunnel to the hibernaculum, and this resulted in one snake sitting in the entrance, and running the recorder until it reached the max-

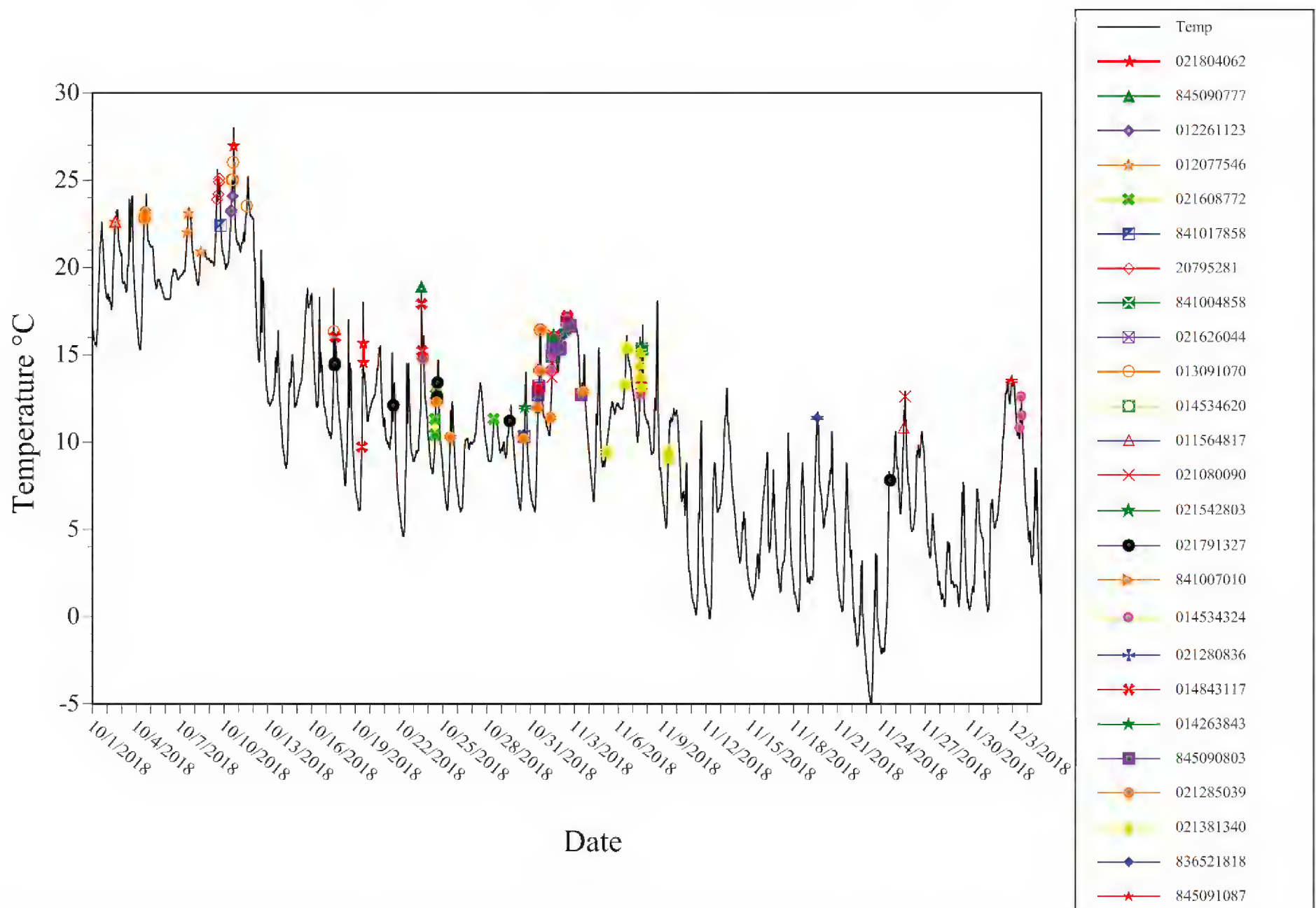


Fig. 3. All activity of snakes at four dens in Bass River State Forest in 2018 as a function of date and soil surface temperature.

imum number of data points it could store ($n = 2,500$). Figure 2 also indicates the period when the recorder was not recording. Because of this, the recorder was moved up to the entrance on 9 November 2017 (so that even if a snake was in the tunnel, watching the outside world with its head at the entrance, it would not record the activity more than a few times). During this down period from 29 October to 9 November some snakes entered (and may have left) without being recorded. Even so, the pattern clearly shows 18 different snakes (ages 0 [hatchling] to 16 years) entering, leaving, and re-entering from 16 October to 17 November 2017. The recorder continued monitoring through December but due to freezing surface temperatures, there was no more activity.

In 2018, the activity of 25 Northern Pine Snakes began on 2 October and continued to 7 December at Bass River State Forest (Fig. 3). The pattern was similar to that in 2017 in that there was daily temperature variation, and the snakes entered and left numerous times. It is, however, important to acknowledge these patterns because they show that activity is rather constant. Thus, the first hypothesis of a restricted time period of activity around the hibernacula was rejected.

Additionally, the Davenport Den (Ocean County) was monitored in the Fall of 2018. In winter of 2017–2018 only one two-year old Northern Pine Snake and two Corn Snakes *Elaphe guttata* (now *Pantherophis guttatus*) used this hibernaculum. In the fall of 2018, it was used by two hatchlings and the same two-year old. There were thus

no large snakes that might influence the activity of the small Northern Pine Snakes; and the hatchlings were extremely active. The activity at this hibernaculum started on 1 November and ended on 28 December 2018. Since there were so few snakes at this hibernaculum, the individual activity patterns of the hatchlings are given in greater detail below.

Time of Day: As might be expected for ectothermic species, snakes were most active during the day and more so on warm sunny days. Most activity occurred between 1000 and 1700 h in both years (Fig. 4). In 2017, 77% of the activity occurred between 1000 and 1600 h; in 2018, 84% of the activity occurred in this same time period. However, in 2017 one snake left at 2000 h, and in 2018, one entered at 2045 h, and another entered at 0100 h at night. These rarely observed nocturnal activities occurred during relatively high temperatures ($> 14^{\circ}\text{C}$).

Temperature effects: The activity of the snakes was plotted against the soil surface temperatures for 2017 and 2018 as a function of date (Figs. 2 and 3). In both years, the October surface temperatures were high, and they generally decreased throughout the fall. Snakes did not enter or leave at the lowest daily temperature (at night), but sometimes entered or left at the highest temperatures for the day. Most of the activity occurred at temperatures of 10°C or above. In both years there was little activity when the surface temperature fell below 8°C at night.

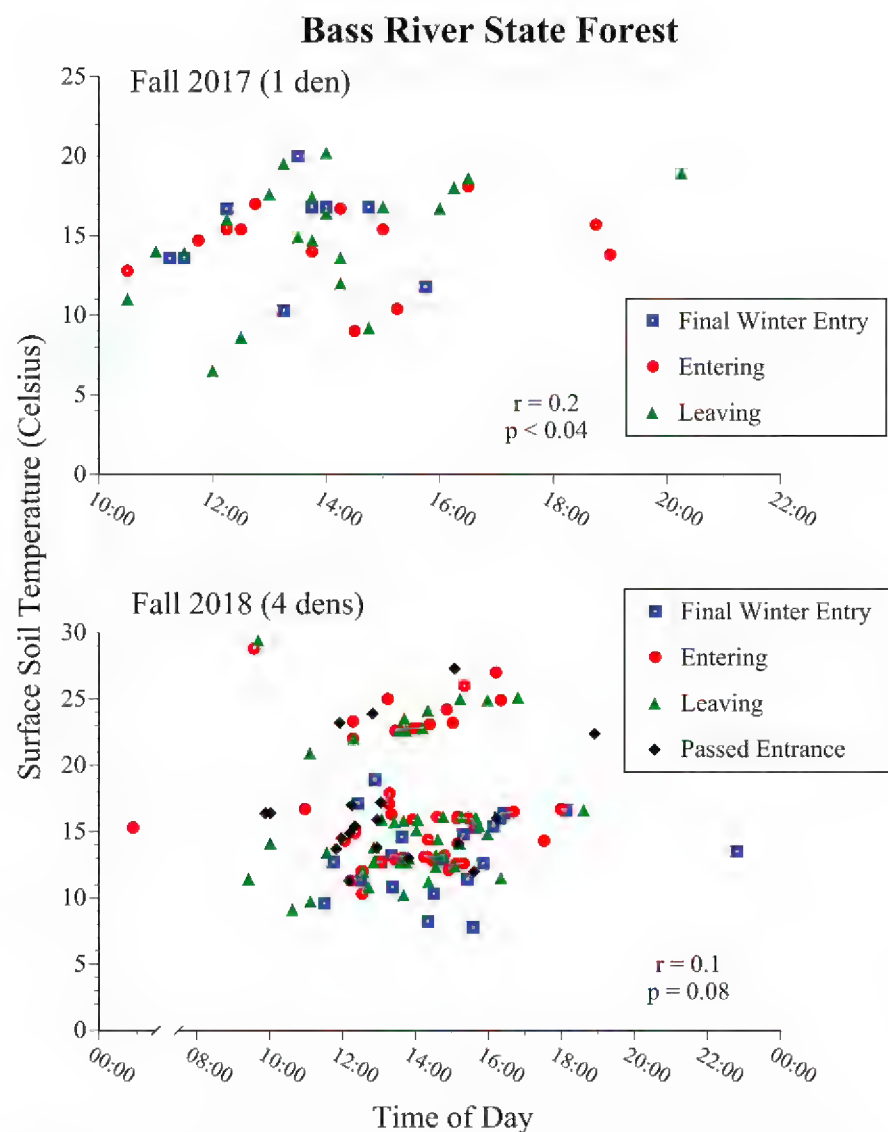


Fig. 4. Fall activity of snakes in Fall 2017 and Fall 2018 as a function of time of day and surface soil temperature. Activity type is noted by each symbol.

However, it is noteworthy that for both years, snakes entered or left the hibernacula, even after prolonged periods of daily low temperatures that reached 0° C in 2017, and even -5° C in 2018 (Figs. 2 and 3).

Although the daily high and low temperatures and snake movements were correlated ($r = 0.54$ in 2017; 0.51 in 2018; both $P < 0.0001$), the daily high and low temperatures for each day were more highly correlated ($r = 0.71$ in 2017; 0.79 in 2018, Fig. 5). Figure 5 indicates when snakes either entered or left a hibernaculum, or made their final entry for the winter. Note that some snakes entered at the same temperature point, and so there are fewer points than snakes. There are fewer points in 2017 because only one hibernaculum was monitored; while the 2018 data refer to all four hibernacula at Bass River State Forest. Final entries were usually at lower temperatures than other activities (Fig. 5).

Individual behavior: Figures 2–3 indicate frequent activity at hibernacula; individual snakes typically entered and left more than once (rejecting the initial hypothesis). The activity of individual snakes was examined only for 2018; individual activity in 2017 was not examined because equipment failure for a short period made it impossible to know whether any snakes left or entered during that period. Some snakes entered a den and remained for the winter (32%), but most did not (68%). At the Bass River study site, some snakes visited all four of the monitored hibernacula on the same day, often returning to the first one they entered. Snakes entered or left hibernacula

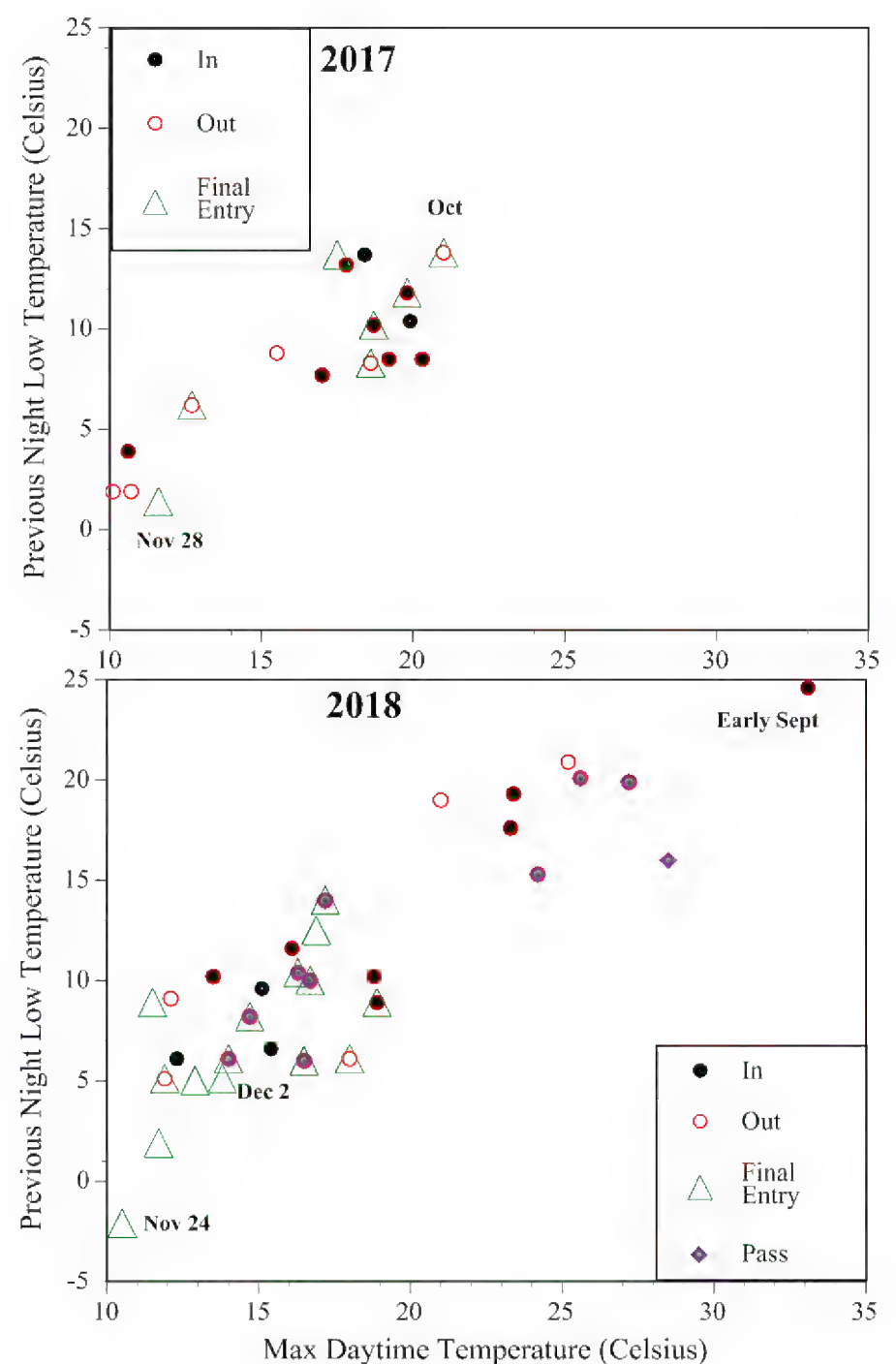


Fig. 5. Activity of snakes in Fall 2017 and Fall 2018 as a function of the maximum daytime temperature and the previous night's low temperature.

an average of 5.6 ± 0.7 times, switched dens an average of 1.4 ± 0.3 times, and visited 1.8 ± 0.2 dens at Bass River State Forest in 2018. Movement was a function of age: older snakes moved more often than younger ones (Table 1). At Bass River State Forest, hatchlings moved an average of only 2.3 ± 1.3 times (Table 1).

However, at the Davenport den, where there were only two hatchlings and one 2-year old, the movement pattern was very different. Hatchlings used the hibernaculum as a home base, and went in and out many times before final entry. Sometimes they remained near the entrance, but they mainly moved a few meters away (e.g., the hatchlings were not immediately located). The seasonal patterns of the two hatchlings are shown in Fig. 6. The two hatchlings moved 48 and 66 times, while the 2-year old moved only 16 times. The lack of older, larger Northern Pine Snakes at the den may have allowed the hatchlings to move more freely.

Activity around each den (entering, leaving) varied significantly ($\chi^2 = 98$, $P < 0.0001$), and the percentage of hits at each den varied: den 1 = 25%, den 2 = 59%, den 4 = 12% and den 5 = 4% of total activity at Bass River. The total activity around the four dens at Bass River State Forest was 139 hits for 25 snakes. At the Davenport den

Table 1. Movement of Northern Pine Snakes among four monitored hibernacula at Bass River State Forest, New Jersey, USA, in 2018.

		Age of Snake (yr)				X ²
		0–1	2–3	5–7	Over 7	
Any Reading	mean	2.3 ± 1.3	2.8 ± 0.9	6.8 ± 2.5	7.1 ± 0.8	8.7 (0.03)
	min/max	1/6	1/5	1/11	3/12	
Den Switches	mean	0.3 ± 0.3	0.5 ± 0.5	1.5 ± 0.7	2.0 ± 0.4	8.0 (0.05)
	min/max	0/1	0/2	0/3	0/5	
Dens Visited	mean	1.3 ± 0.3	1.3 ± 0.3	1.8 ± 0.3	2.2 ± 0.2	7.9 (0.05)
	min/max	1/2	1/2	1/2	1/4	

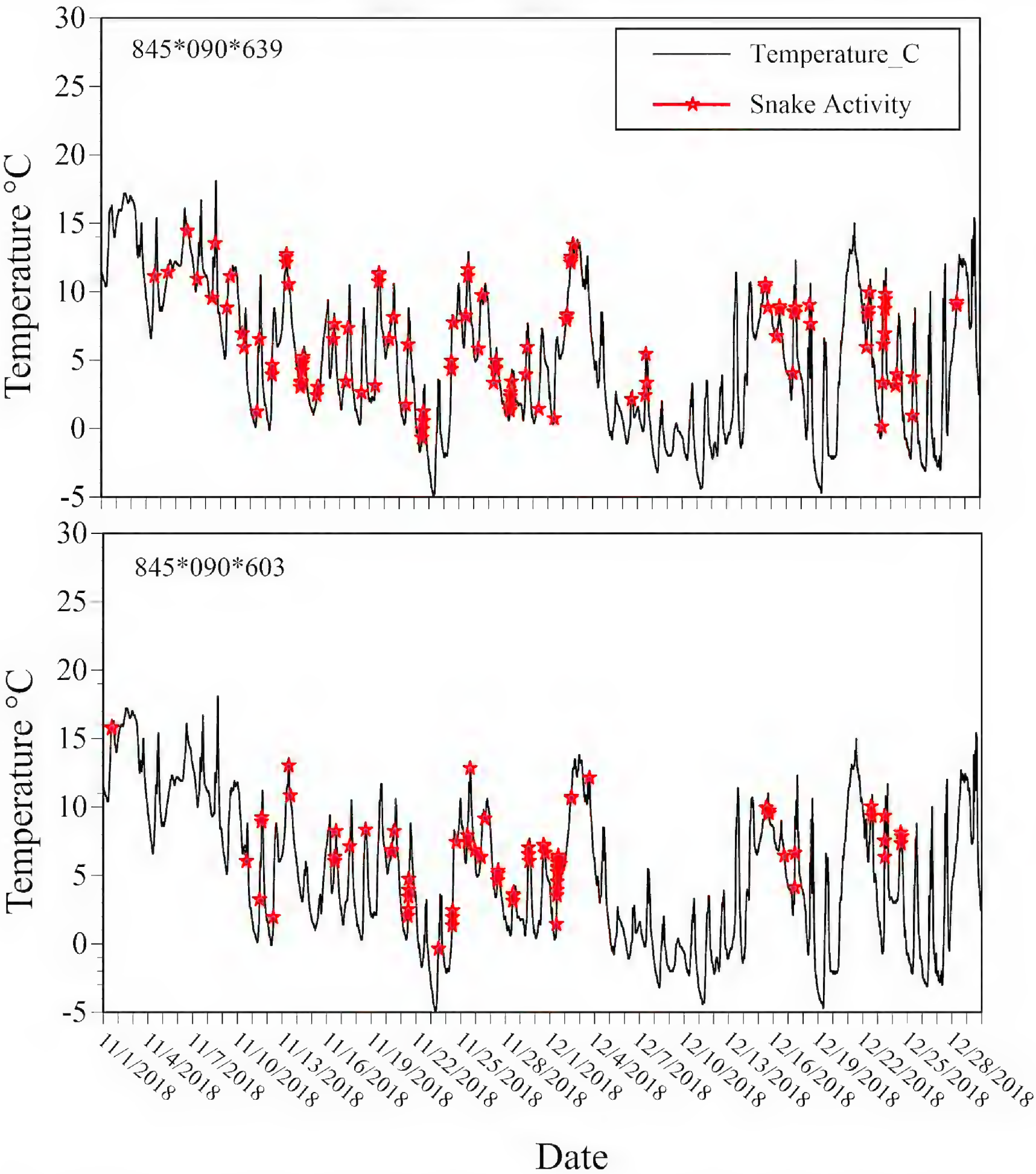


Fig. 6. Activity of two hatchlings (tag numbers 845090639 and 845090603) in the fall of 2018 at den (Davenport) as a function of date and soil surface temperature.

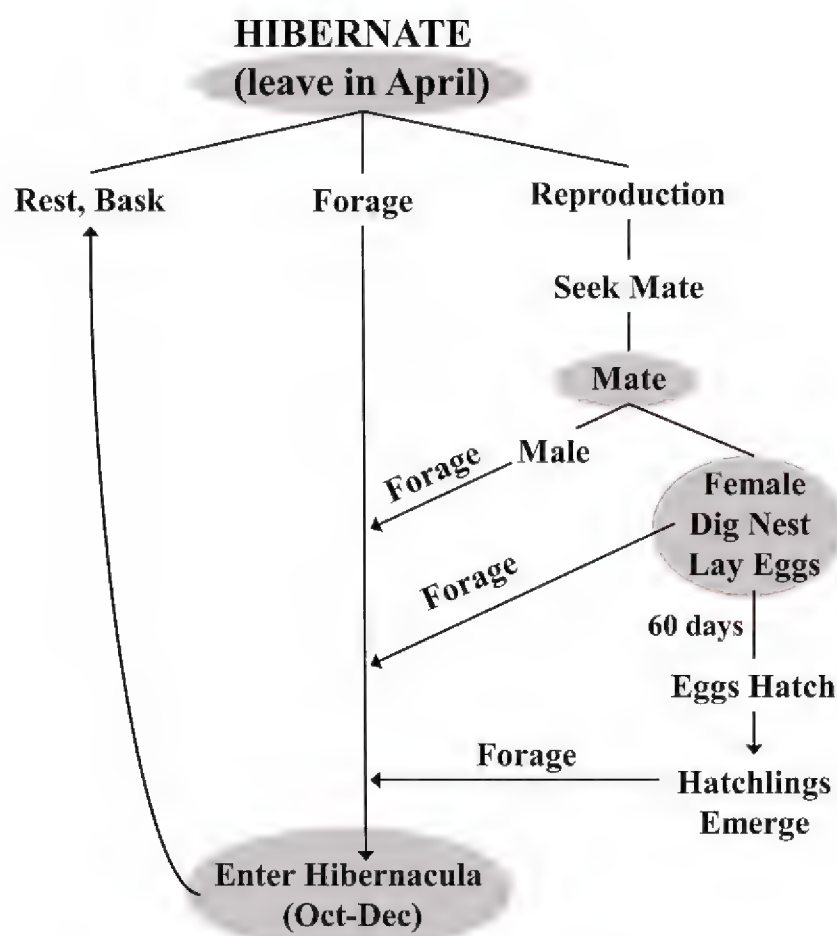


Fig. 7. Schematic of life cycle of Northern Pine Snakes, indicating periods of high vulnerability to human disturbances, such as fire, off-road vehicles, hunting, and poaching.

it was 120 hits for only three snakes, and the two hatchlings accounted for most of this activity.

Discussion

Methodological issues and using the Power Tracker

VIII: Any study of animals in the wild is fraught with variability and uncertainties in the methods used, in environmental variation, and in the behavior and ecology of the species. Data from 2017 indicated that the PIT-tag recording devices could be used in the field with 12-volt marine batteries, since they operated properly, and the data could be retrieved. However, the main problem encountered initially related to placement of the receiver – when it was partway down the hibernaculum entrance, it recorded continuously as some snakes simply rested in the tunnel, peering out of the entrance and filling all the available data points. When the coil was moved to the front of the entrance, this problem did not exist, but then it was difficult to determine if a snake merely passed by the entrance, or entered. This issue could be partly managed by seeing where the individually marked Northern Pine Snake turned up next. The main difficulty with the Power Tracker was that the batteries need to be changed to allow charging every 2–3 weeks depending upon temperature (battery life was shorter at cold temperatures). Batteries need to be charged on the “slow setting” rather than the “rapid method,” as the former provided a longer-lasting charge in the field. Bad weather, heavy rains and snow, and downed trees from severe storms made getting to the study site to change the (20 kg!) batteries every couple of weeks very challenging.

Poaching of Northern Pine Snakes is known to be a major threat (Burger and Zappalorti 2016), so I opted not

to use solar power or place cameras that might call attention to the den entrance. Visible solar panels would alert poachers or vandals to the exact location of hibernacula entrances, and would also encourage theft. Protecting the equipment is important since each set-up costs about \$3,000 for the Tracker, leads, batteries, plastic case for the recorder and batteries, and a wood cover to prevent excessive rain from entering the plastic case. The continuously recording thermometers were only \$25, and the manufacturer’s battery lasted at least a year. Lastly, the tracker should be put in place when the snakes are underground to ensure that they leave a scent trail when they leave so that other snakes (particularly hatchlings) can find the entrance.

With any field study there are weather-related and other environmental variables that can influence the behavior of the snakes. Exceptionally warm weather in 2018 resulted in an extended period of ingress into the hibernacula. No snake entered or left den 1 in 2017 (the only den monitored that year) after 17 November, but in 2018 snakes continued to move in and out of the five dens monitored into late December.

Finally, there are uncertainties that relate to the behavior and ecology of the snakes. These uncertainties were related to age, sex, and individual responses. Age clearly entered as a factor in explaining the observed Northern Pine Snake behavior, but this would not have been clear if the snakes were not of known ages. The behavior of hatchlings varied depending upon the composition of the hibernaculum community (see below). Hatchlings moved very little when they were part of a community that included snakes of different ages (and sizes), but hatchlings moved often when there were no larger (older) snakes present.

Activity patterns around hibernacula in the fall:

Northern Pine Snakes in the present study were very active around the hibernacula for over two months. At Bass River State Forest, snakes moved in and out an average of six times, often switching dens. The hypotheses that activity around a hibernaculum was restricted in time, and that snakes entered a hibernaculum and stayed were both rejected. Individuals moved from den to den over a few days or a few weeks, partly dependent upon weather. That Northern Pine Snakes left a given den and did not enter another den for several days, but then returned, indicates that there are some other suitable places to shelter when the temperatures drop at night. Nonetheless, the snakes came back to one of their original dens when the weather warmed up enough to move.

The factors that played a role in activity were season (date), temperature (daytime high and nighttime low), age, and hibernaculum number. The Northern Pine Snakes appeared to prefer two of the dens over the others. However, even snakes that first entered one of the “preferred dens” moved to other dens before returning. One might predict that older snakes, aware of the advantages and disadvantages of one den over another, might

move less than young snakes that are less familiar with their environment and den options. This, however, was not the case. The reason for increased switching with increasing age is unclear. However, in the absence of larger (older) snakes, the two hatchlings at the Davenport den (an isolated hibernaculum) used it as a home base, and moved in and out frequently, depending upon temperature. These two hatchlings did not finally enter for the winter until 28 December.

Factors affecting entry into hibernacula: Clearly there are seasonal and temperature effects; Northern Pine Snakes enter hibernacula to avoid freezing winter temperatures, as do other snakes in northern climates. Although other studies have examined the temperatures of snakes during hibernation (Costanzo 1986; Hein and Guyer 2009), or emergence in the spring (Todd et al. 2009), little is known about the temperatures at which snakes enter hibernacula in the fall. To study fall behavior requires: 1) individually marked snakes, 2) a method of recording each snake's entrance in the fall (date, time of day), and 3) devices to continuously record the soil surface temperature to capture the temperature when snakes enter. A long-term study was required for the first criterion, and recent technological developments were required for the latter two. The development of this new technology makes it possible to more accurately examine both the seasonal and temperature influences on snakes entering and emerging from hibernacula, as well as indicating the degree to which snakes move in and out during both entry and emergence, before finally dispersing in the spring to forage, mate, and nest.

In this study, date and sand surface temperature determined when snakes entered and left hibernacula, and snakes moved an average of about six times before settling in for the winter. Three other factors seemed to also affect movement: den number, age of the snakes, and for hatchlings, the presence of older (larger) snakes. Snakes clearly preferred two of the monitored dens over the other two, and both of the preferred dens were deeper than the other two, and they were older in terms of usage history (Burger and Zappalorti 2011).

The age of the snakes influenced their movement; older snakes moved more often, switched dens more often, and visited more dens than did younger snakes. This was unexpected since younger snakes might be expected to explore a range of different sites before settling down. Hatchlings using the four hibernacula at the Bass River State Forest showed significantly less activity than older snakes (an average of only two movements/snake). However, the two hatchlings that used the Davenport hibernaculum showed activity 48 and 66 times. They not only entered and left (and were not visible around the hibernaculum or in the surrounding area), but sometimes basked very near the entrance, moving swiftly down the entrance when approached by the researchers. They appeared to be using the hibernaculum as a refuge and an

overnight site for nearly two months before remaining for the winter (refer to Fig. 6). This difference in hatchling behavior at the two sites may relate to the relative risk posed by much larger snakes using the same hibernaculum. If large, heavy (up to 1,350 g) adult snakes are entering and leaving, they pose a risk to hatchlings (30–50 g), and adults could injure them while both are moving through the tunnels. The only dead snakes found in hibernacula over the years (with one exception of small mammal predation) were those squashed flat by older, larger snakes lying on top of them for long periods of time.

Vulnerability, risk, and competing claims: Northern Pine Snakes are most vulnerable when they are roaming above ground (even though they are partially fossorial), and when they are concentrated in one small area. They are above ground at intermediate temperatures; in the hot summer they spend a great deal of time in hollow fallen logs, under leaves and needles, or underground; in the winter they hibernate 1–2 m below ground. Behaviorally they are vulnerable when they are mating (spatially scattered), nesting (females, spatially clumped), and entering or leaving hibernacula (clumped around hibernacula). The vulnerability of Northern Pine Snakes is greatest when these two features (above ground and clumped) overlap, which occurs when entering hibernacula for the winter. This situation occurs when entering hibernacula for all Northern Pine Snakes, and for females when they are nesting (Burger and Zappalorti 1992, 2011, 2016; Burger et al. 2017, 2018, Fig. 7). The data presented in this paper clearly show that the period of ingress into hibernacula is at least two months in duration, spanning both October and November, and can extend through December if the weather is not too cold. The data also show that there is frequent activity, not just one entry into the hibernaculum by each snake. The dens at Bass River State Forest that were studied are about 30–120 m from each other. That snakes come and go indicates that the spatial area of activity is greater than just around the immediate entrance to a hibernaculum.

Only once was a Northern Pine Snake seen above ground in the fall, although later analysis of the recorded data indicated that just minutes before or after our presence, snakes entered or left the hibernaculum. In one case, a snake came up five minutes after we finished downloading the data, and I only saw it because I went back to pick up a piece of equipment. It was lying in the tunnel, with its head about 2 cm from the entrance. This observation emphasizes the importance of having continuously recording equipment; observation alone would not yield this key information.

The major risks that Northern Pine Snakes face are natural predators (hawks, mammals, and other ophiophagus snake species), commensal predators (dogs, raccoons), poachers, loss of habitat, and human disturbance (direct and indirect). Human disturbance can take the form of people disrupting snake behavior (e.g., dur-

ing snake copulation, nesting, or entering/leaving hibernacula), disrupting snake habitat (e.g., off-road vehicles, ORV races through the Pine Barrens, prescribed burns, tree-cutting), or a combination of these activities. Many of these activities represent competing claims for the same Pine Barrens habitat (either quantity or quality of the habitat).

The resolution of competing claims for Pine Barrens habitat, or management of that habitat, is partly a societal decision. However, the specific needs of wildlife, and of specific species such as the Northern Pine Snake, cannot be considered unless there are data to show what those needs are, when (and what) their vulnerabilities are, and when interference will jeopardize their populations. In many cases, a situation can be resolved by bringing together the relevant people and agencies, and determining the best course of action. Clearly, debris and dry leaves that will result in a hot fire that could destroy local human communities or businesses (as well as wildlife) need to be reduced, and fire management is a reasonable option. Likewise, hunting, ORV races, and other human activities are reasonable uses of public forests such as the Pine Barrens. Protection of endangered and threatened species is another public goal (as well as being a legal one) that must be considered. Even accepting the latter as an important public goal does not completely solve the problem, however, because different species may have different requirements and vulnerable periods. For each species, relative abundance and total distribution need to be considered, with species which have very restricted ranges getting priority treatment. A consensus needs to be reached both about the specific habitat and ecological requirements of different endangered, threatened, or otherwise vulnerable species, and about the specific requirements of other groups with competing claims (e.g., foresters, recreationists, fire managers). Armed with this knowledge, managers can make science-based, societally-based, and cost-effective decisions about managing the habitat and the associated wildlife species that occur there.

Conclusions

Plants and animals in the Pine Barrens, and everywhere else, face competing claims to their habitat along with the risks of disruption or disturbances from other animals, poachers, recreationists, foresters, resource managers, firemen, developers, and the general public. Resolving competing claims requires having knowledge of the specific needs of vulnerable species and habitats, as well as the needs of the people and managers. One high risk vulnerable period for Northern Pine Snakes is when they enter or leave their winter hibernation sites. This paper provides data showing that the fall ingress period to hibernacula is prolonged (over two months), and involves frequent snake activity above ground. During October and November in the Pine Barrens, Northern Pine Snakes

are moving toward hibernacula, concentrating there, and entering and leaving frequently until they eventually stay underground for the winter. Thus, this is a highly vulnerable period when snakes are concentrated, and any disruptions (such as fires or off-road vehicle races) have the potential to injure or kill Northern Pine Snakes that are threatened in New Jersey, and threatened or endangered throughout most of their range. New Jersey has perhaps the largest, and most stable population of Northern Pine Snakes throughout the range of the species (Golden et al. 2009; Burger et al. 2016, 2017), therefore state wildlife agencies have a special responsibility to ensure its continued survival.

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Literature Cited

- Anderson CD. 2010. Effects of movement and mating patterns on gene flow among overwintering hibernacula of Timber Rattlesnake (*Crotalus horridus*). *Copeia* 2010: 54–61.
- Andrews KM, Gibbons JW, Jochimsen DM, Mitchell J. 2008. Ecological effects of roads on amphibians and reptiles: a literature review. *Herpetological Conservation* 3: 121–143.
- Blouin-Demers G, Weatherhead PJ. 2001. Habitat use by Black Rat Snakes (*Elaphe obsoleta obsoleta*) in fragmented forests. *Ecology* 82: 2,882–2,896.
- Burger J, Zappalorti RT. 1992. Philopatry and nesting phenology of Pine Snakes *Pituophis melanoleucus* in New Jersey Pine Barrens. *Behavioral Ecology and Sociobiology* 30: 331–336.

- Burger J, Zappalorti RT. 2011. *The Northern Pine Snake (Pituophis melanoleucus) in New Jersey: Its Life History, Behavior and Conservation*. Nova Science Publishers, New York, New York, USA. 92 p.
- Burger J, Zappalorti RT. 2015. Hibernation site philopatry in Northern Pine Snakes (*Pituophis melanoleucus*) in New Jersey. *Journal of Herpetology* 49: 245–251.
- Burger J, Zappalorti RT. 2016. Conservation and protection of threatened Pine Snakes (*Pituophis melanoleucus*) in the New Jersey Pine Barrens, USA. *Herpetological Conservation and Biology* 11: 304–314.
- Burger J, Zappalorti RT, Gochfeld M, Boarman W, Caffrey M, Doig V, Garber S, Mikovsky M, Safina C, Saliva J. 1988. Hibernacula and summer dens of Pine Snakes (*Pituophis melanoleucus*) in the New Jersey Pine Barrens. *Journal of Herpetology* 22: 425–433.
- Burger J, Zappalorti RT, Gochfeld M. 2000. The defensive behaviors of Pine Snakes (*Pituophis melanoleucus*) and Black Racers (*Coluber constrictor*) to disturbance during hibernation. *Herpetological Natural History* 7: 59–66.
- Burger J, Zappalorti RT, Gochfeld M, Burket D, Schneider D, McCort D, Jeitner C. 2012. Long-term use of hibernaculum by Northern Pine Snakes (*Pituophis melanoleucus*). *Journal of Herpetology* 46: 596–601.
- Burger J, Gochfeld M, Zappalorti RT, DeVito E, Jeitner C, Pittfield T, Schneider D, McCort M. 2017. Stakeholder contribution to conservation of threatened Northern Pine Snakes (*Pituophis melanoleucus* Daudin, 1803) in the New Jersey Pine Barrens as a case study. *Amphibian & Reptile Conservation* 11(2) [General Section]: 17–32 (e142).
- Burger J, Zappalorti RT, Gochfeld M. 2018. Hatchling survival to breeding age in Northern Pine Snakes (*Pituophis melanoleucus*) in the New Jersey Pine Barrens: human effects on recruitment from 1986 to 2017. *PloS One* 13: e0195676.
- Cardillo M, Mace GM, Gittleman JL, Jones KE, Bielby I, Purvis A. 2008. The predictability of extinction: biological and external correlates of decline in mammals. *Proceedings of the Royal Society Biological Science* 275: 1,441–1,448.
- Clark RW, Brown WS, Stechert R, Zamudio KR. 2008. Integrating individual behavior and landscape genetics: the population structure of timber rattlesnake hibernacula. *Molecular Ecology* 17: 719–730.
- Costanzo JP. 1986. Influences of hibernaculum micro-environment on the winter life history of the garter snake (*Thamnophis sirtalis*). *Ohio Journal of Science* 86: 199–204.
- Croak BM, Crowther MS, Webb JK, Shine R. 2013. Movements and habitat use of an endangered snake *Hoplocephalus bungaroides* (Elapidae): implications for conservation. *PloS One* 8: e61711.
- Forman RTT, Godron M. 1986. *Landscape Ecology*. Wiley, New York, New York, USA. 619 p.
- Gaiara MP, Alencar LRV, Valujo PH, Tambosi R, Martins M. 2015. Setting conservation priorities within monophyletic groups: an integrative approach. *Journal of Nature Conservancy* 24: 49–55.
- Gerald GW, Bailey MA, Holmes JN. 2006. Habitat utilization of *Pituophis melanoleucus* (Northern Pine Snakes) on Arnold Air Force Base in Middle Tennessee. *Southeastern Naturalist* 5: 253–264.
- Gibbons JW, Scott DE, Ryan TJ, Buhlmann DA, Tuberville TD, Mettsm B, Greene JL, Mills TM, Leiden YA, et al. 2000. The global decline of reptiles, *Déjà vu* amphibians. *BioScience* 50: 653–666.
- Golden DM, Winkler P, Woerner P, Fowles G, Pitts W, Jenkins D. 2009. *Status Assessment of the Northern Pine Snake (Pituophis m. melanoleucus) in New Jersey: An Evaluation of Trends and Threats*. New Jersey Department of Environmental Protection, Division of Fish and Wildlife, Endangered and Nongame Species Program, Trenton, New Jersey, USA. 53 p.
- Gregory PT. 1974. Patterns of spring emergence of the Red-sided Garter Snake (*Thamnophis sirtalis parietalis*) in the Interlake region of Manitoba. *Canadian Journal of Zoology* 52: 1,063–1,069.
- Harvey DA, Weatherhead PJ. 2006. Hibernation site selection of Eastern Massasauga Rattlesnakes (*Sistrurus catenatus catenatus*) near their northern range limit. *Journal of Herpetology* 40: 66–73.
- Hein AM, Guyer C. 2009. Body temperatures of overwintering cottonmouth snakes: hibernaculum use and inter-individual variation. *Journal of the Alabama Academy of Science* 80: 35–44.
- Hilton-Taylor C. 2000. *2000 IUCN Red List of Threatened Species*. International Union for the Conservation of Nature, Gland, Switzerland. 61 p.
- Hirth HF, Pendleton RC, King AC, Downard TR. 1969. Dispersal of snakes from a hibernaculum in northwestern Utah. *Ecology* 50: 332–339.
- IUCN; UNEP. 2009. *The World Database on Protected Areas (WDPA)*. United Nations Environment World Conservation Monitoring Centre, Cambridge, United Kingdom. Available: <https://www.protectedplanet.net/> [Accessed: 28 December 2018].
- Joy JE, Crews D. 1987. Hibernation of garter snakes (*Thamnophis sirtalis parietalis*): seasonal cycles of cold tolerance. *Comparative Biochemistry and Physiology* 87A: 1,097–1,101.
- Kapfer JM, Pekar CW, Reineke DM, Conggins JR, Hay R. 2010. Modeling the relationship between habitat preferences and home-range size: a case study on a large mobile colubrid snake from North America. *Journal of Zoology* 282: 13–20.
- Kjoss VA, Litvaitis JA. 2001. Community structure of snakes in a human-dominated landscape. *Biological Conservation* 98: 285–292.
- McLeod RF, Gates JE. 1998. Responses of herpetofaunal communities to forest cutting and burning at Chesapeake Farms, Maryland. *American Midland Naturalist* 139: 164–177.

- MWPARC. 2009. *Prescribed Fire Use and Important Management Considerations for Amphibians and Reptiles within the Midwest*. Midwest Partners in Amphibian and Reptile Conservation. Available: <http://www.mwparc.org/> [Accessed: 30 December 2018].
- Parent C, Weatherhead PJ. 2000. Behavioral and life history responses of Eastern Massasauga Rattlesnake (*Sistrurus catenatus catenatus*) to human disturbance. *Oecologia* 125: 170–178.
- Pimm SL, Raven P. 2000. Biodiversity: extinction by numbers. *Nature* 430: 843–845.
- Pimm SL, Russell GI, Gittleman IL, Brooks TM. 1995. The future of biodiversity. *Science* 269: 347–350.
- Pimm SL, Ayres M, Balmford A, Branch G, Brandon K, Brooks K, Bustamante R, Costanza R, Cowling R, Curran LM, et al. 2001. Can we defy nature's end? *Science* 293: 2,207–2,208.
- Polasky S. 2008. Why conservation planning needs socioeconomic data. *Proceedings of the National Academy of Sciences of the United States of America* 105: 6,505–6,506.
- Roth L, Franklin R. 2018. *Timing of Prescribed Fire in Longleaf Pine Management: Benefits, Risks, and Roles by Season*. Clemson University, Forestry Leaflet 32. Clemson University, Clemson, South Carolina, USA. Available: http://www.southernfireexchange.org/SFE_Publications/etc/Clemsonforfl32.pdf [Accessed: 30 December 2018].
- Rudolph DC, Schaefer RR, Burgdorf SJ, Duran M, Conner RN. 2007. Pine Snake (*Pituophis ruthveni* and *P. melanoleucus lodingi*) hibernacula. *Journal of Herpetology* 41: 560–565.
- Sanderson EW, Jaiteh M, Levy MA, Redford KH, Wannebo AV, Woolmer G. 2002. The human footprint and the last of the wild. *BioScience* 52: 891–904.
- Segura C, Feriche M, Pleguezuelos JM, Santos X. 2007. Specialist and generalist species in habitat use: implications for conservation assessment in snakes. *Journal of Natural History* 41: 2,765–2,774.
- Semlitsch RD, Bodie JR. 2003. Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conservation Biology* 17: 1,219–1,228.
- Shine R, Langkilde T, Wall M, Mason RT. 2006. Temporal dynamics of emergence and dispersal of garter snakes from a communal den in Manitoba. *Wildlife Research* 33: 103–111.
- Steen DA, McGee AER, Hermann SM, Stiles JA, Guyer C. 2010. Effects of forest management on amphibians and reptiles: generalist species obscure trends among native forest associates. *Open Environmental Service* 4: 24–30.
- Tanentzap AJ, Walker S, Stephens RTT, Lee WG. 2012. A framework for predicting species extinction by linking population dynamics with habitat loss. *Conservation Letters* 5: 149–156.
- Todd BD, Andrews KM. 2008. Response of a reptile guild to forest harvesting. *Conservation Biology* 22: 753–761.
- Todd J, Amiel J, Wassersug R. 2009. Factors influencing the emergence of a northern population of Eastern Ribbon Snakes (*Thamnophis sauritus*) from artificial hibernacula. *Canadian Journal of Zoology* 87: 1,221–1,226.
- Wilcove D, Rothstein D, Dubow J, Phillips A, Losos E. 2000. Leading threats to biodiversity: what's imperiling US species. Pp. 239–254 In: *Precious Heritage: The Status of Biodiversity in the United States*. Editors, Stein BA, Kutner LS, Adams JS. Oxford University Press, New York, New York, USA. 399 p.
- Williams KE, Hodges KE, Bishop CA. 2012. Small reserves around hibernation sites may not adequately protect mobile snakes: the example of Great Basin Gopher Snakes (*Pituophis catenifer deserticola*) in British Columbia. *Canadian Journal of Zoology* 90: 304–312.
- Wilson EO. 1992. *The Diversity of Life*. W.W. Norton, New York, New York, USA. 424 p.
- Wilson HB, Joseph LN, Moore AL, Possingham HP. 2011. When should we save the most endangered species? *Ecology Letters* 14: 886–890.
- Wilson KA, Carwardine J, Possingham HP. 2009. Setting conservation priorities. *Annals of the New York Academy of Sciences* 1162: 237–264.
- Zappalorti RT, Burger J, Burkett DW, Schneider DW, McCort MP, Golden DM. 2014. Fidelity of Northern Pine Snakes (*Pituophis m. melanoleucus*) to natural and artificial hibernation sites in the New Jersey Pine Barrens. *Journal of Toxicology and Environmental Health Part A* 77: 1,285–1,291.



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From incidental findings to systematic discovery: locating and monitoring a new population of the endangered Harlequin Toad

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Abstract.—The scientific and conservation communities recognize toads of genus *Atelopus* as among the most vulnerable of all amphibian groups, with over 75% of the species assessed as “Critically Endangered” by the International Union for the Conservation of Nature (IUCN) Red List. *Atelopus varius*, known as the Harlequin Toad, has been thought to be extinct in Costa Rica since the mid-1990s. There have been four rediscovered populations of the species since 2004. This report presents the fifth reappearance of *A. varius*, this time in the Alexander Skutch Biological Corridor (ASBC) in the Pacific Slope foothills of La Amistad International Park, Costa Rica, which represents a new location. Previously, the pattern of reappearance of this species has been unclear. In this study, the discovery of a new population of *A. varius* allows us to evaluate the presence of *Bd* infection and offer critical natural history remarks. In total, 25 different individuals were identified. All samples analyzed for *Bd* diagnosis were negative. In contrast to other *A. varius* populations, this one was mostly found high above the riverbed, often in the foliage, tree trunks, and bromeliads, from 1–6 m above the water both during day and night. The absence of *Bd* infection in these Harlequin Toads, a highly susceptible species, in an area identified as having a high probability of *Bd* occurrence, suggests that this behavior could have helped this population survive by reducing infection risk. Moreover, the distribution of *A. varius* may have changed in the last 50 years, by penetrating higher in the montane regions of the Talamanca mountains, a change in distribution that might also help its survival of some environmental stressors. With the discovery of a new locality for *A. varius*, this study offers an animal behavior argument to account for species recovery in general, as well as a possible expansion of what has been accepted as the historical distribution of this species.

Keywords. *Atelopus varius*, natural history, citizen science, endangered species, chytrid fungus, Alexander Skutch Biological Corridor, Costa Rica

Resumen.—Los sapos del género *Atelopus* son reconocidos como uno de los grupos de anfibios más vulnerables, con más del 75 por ciento de las especies de este género evaluadas como “En Peligro Crítico” por la Lista Roja de la Unión Internacional para la Conservación de la Naturaleza. Se pensaba que *Atelopus varius*, conocido como la rana Arlequín, se había extinguido en Costa Rica desde mediados de los años noventa. Después de 2004 ha habido cuatro redescubrimientos de la especie. Este informe presenta la quinta reaparición de *A. varius*, esta vez en el Corredor Biológico Alexander Skutch (ASBC) en las estribaciones de la vertiente del Pacífico del Parque Internacional La Amistad, Costa Rica como una nueva ubicación. Hasta ahora, el patrón de reaparición de esta especie no ha sido claro. En este estudio, con el descubrimiento de una nueva población de *A. varius*, ofrecemos importantes observaciones de historia natural y evaluamos la presencia de la infección por *Bd*. En total, se identificaron 25 individuos diferentes. Todas las muestras analizadas para el diagnóstico de *Bd* fueron negativas. En contraste con otras poblaciones de *A. varius*, en nuestro caso se encontró la mayoría de los individuos alto sobre el lecho del río, a menudo en el follaje, troncos de árboles y bromelias, entre 1–6 m sobre el agua, tanto de día como de noche. La ausencia de infección por *Bd* en estas ranas arlequín, una especie altamente susceptible, en un área identificada como con una alta probabilidad de ocurrencia de *Bd*, sugiere que los sapos que pasen menos tiempo cerca del río y más tiempo en áreas abiertas, podría haber ayudado a esta población a sobrevivir mediante la reducción al riesgo de infección. Además, hipotetizamos que la distribución de *A. varius* pudo haber cambiado en los últimos 50 años, penetrando más alto en las regiones montañas de la cordillera de Talamanca, un cambio en la distribución que también podría

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estar ayudando con su supervivencia a ciertos factores ambientales. Con el descubrimiento de una nueva localidad para *A. varius*, nuestro estudio ofrece un argumento de comportamiento animal para explicar la recuperación de especies, así como una posible expansión de lo que se ha aceptado como la distribución histórica de esta especie.

Palabras clave. *Atelopus varius*, historia natural, ciencia ciudadana, reaparición de especies amenazadas, rana arlequín, hongo quitrido, Corredor Biológico Alexander Skutch, Costa Rica

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Introduction

Over 75% of the species in genus *Atelopus* are assessed as “Critically Endangered” by the International Union for the Conservation of Nature Red List (IUCN 2015). Within this genus, the Harlequin Toad (*Atelopus varius*, in Spanish as “Rana Harlequín”), is the last remaining species in Costa Rica. The other three representatives are believed to be extinct (Barrio-Amorós and Abarca 2016).

This moderately-sized toad is associated with small, fast-moving streams, where it is found along the banks and sitting on rocks in the stream. Endemic to Costa Rica and Panama, at one point there were over 100 known populations in both the Atlantic and Pacific Slope versants of the mountain ranges in Costa Rica and western Panama, reaching up to 2,000 m asl (Savage 2002). In the mid-1990s, *A. varius* was believed to be extirpated from Costa Rica, following a drastic decline that began in Monteverde in 1988, where it remains absent (Pounds and Crump 1994). Apart from habitat loss, two other leading possible explanations for the Harlequin Toad’s disappearance are climate variations and fungal disease. Research so far suggests the possibility that the decline of *A. varius* has a multifactor explanation (Pounds et al. 2010; Berger et al. 1998) combining both the effect of climate stress and the appearance of the fungus *Batrachochytrium dendrobatidis* (*Bd*).

Since its loss during the 1990s, four known re-discoveries of the species have been documented, each in private properties in the Pacific slope in Puntarenas Province, Costa Rica. In 2003, a population was discovered at Fila Chonta, Quepos (Pounds et al. 2010; Ryan et al. 2005). Nine years later, to the South, a new breeding population was found near Las Tablas, near the Panamanian border, at 1,300 m asl (González-Maya et al. 2013). One individual of *A. varius* was reported near Buenos Aires, at an elevation of 840 m asl (Solano-Cascante et al. 2014). Moreover, there was a recent rediscovery of *A. varius* by Barrio-Amorós and Abarca (2016) at 400 m asl in a rocky stream located in the Uvita Region. Those authors observed nine adult males, one of which was dead. They tested two live individuals and the one dead specimen for *Bd*, and the dead specimen was the only one to test positive for *Bd* (Barrio-Amorós and Abarca 2016).

The pattern of previous reappearances of this species has been unclear. In some cases, it appeared in small

secluded creeks (Barrio-Amorós and Abarca 2016), and in others, it appeared in large, open, and fast flowing rivers (Gonzalez-Maya et al. 2013; Solano-Cascante et al. 2014), and at altitudinal ranges from almost sea level to as high as 1,500 m asl. The sites identified thus far are all on the Pacific slope, amid degraded landscapes in unprotected land and fragmented, isolated conditions. This pattern of reappearance makes these populations highly vulnerable to habitat loss and environmental risks, such as the population at one of the known localities in Costa Rica which is under serious threat of landslides (Pounds et al. 2010).

Here a newly discovered population of *A. varius* is presented, found in the Alexander Skutch Biological Corridor (ASBC), in the Pacific Slope foothills of La Amistad International Park. We present crucial natural history remarks and evaluate the presence of *Bd* infection in this population. The natural history of amphibian species can hold important clues for their survival, given that the effect of fungal infection can be modified by the host’s ecology, behavior, and life history (Woodhams et al. 2008). The exact location of the site is withheld, given that the protection of such sensitive information is of utmost importance in the case of *A. varius* in Costa Rica. All the re-discovered populations are outside of national protected areas and hence are highly susceptible to commercial trade, collection, and the irresponsible handling that can lead to a decrease in population or increased risk of disease. The IUCN guidelines acknowledge this risk regarding sensitive data access and the publishing of data that identify specific geographic locations (IUCN 2012).

Materials and Methods

The ASBC extends over 6,012.60 ha (Fig. 1) within the canton of Pérez Zeledón in the province of San José. The principal communities of the ASBC include Quizarrá, Santa Elena, Montecarlo, San Francisco, San Ignacio, Santa Marta, Santa María, and Trinidad. The ASBC lies between 650 and 1,650 m asl and contains the first remnant population of *A. varius* outside the province of Puntarenas.

Note that the findings documented by community members and students of the Faculty of Environmental Studies of York University were essential in locating this population of *A. varius*. The incidental sightings came from at least three separate locations along two different

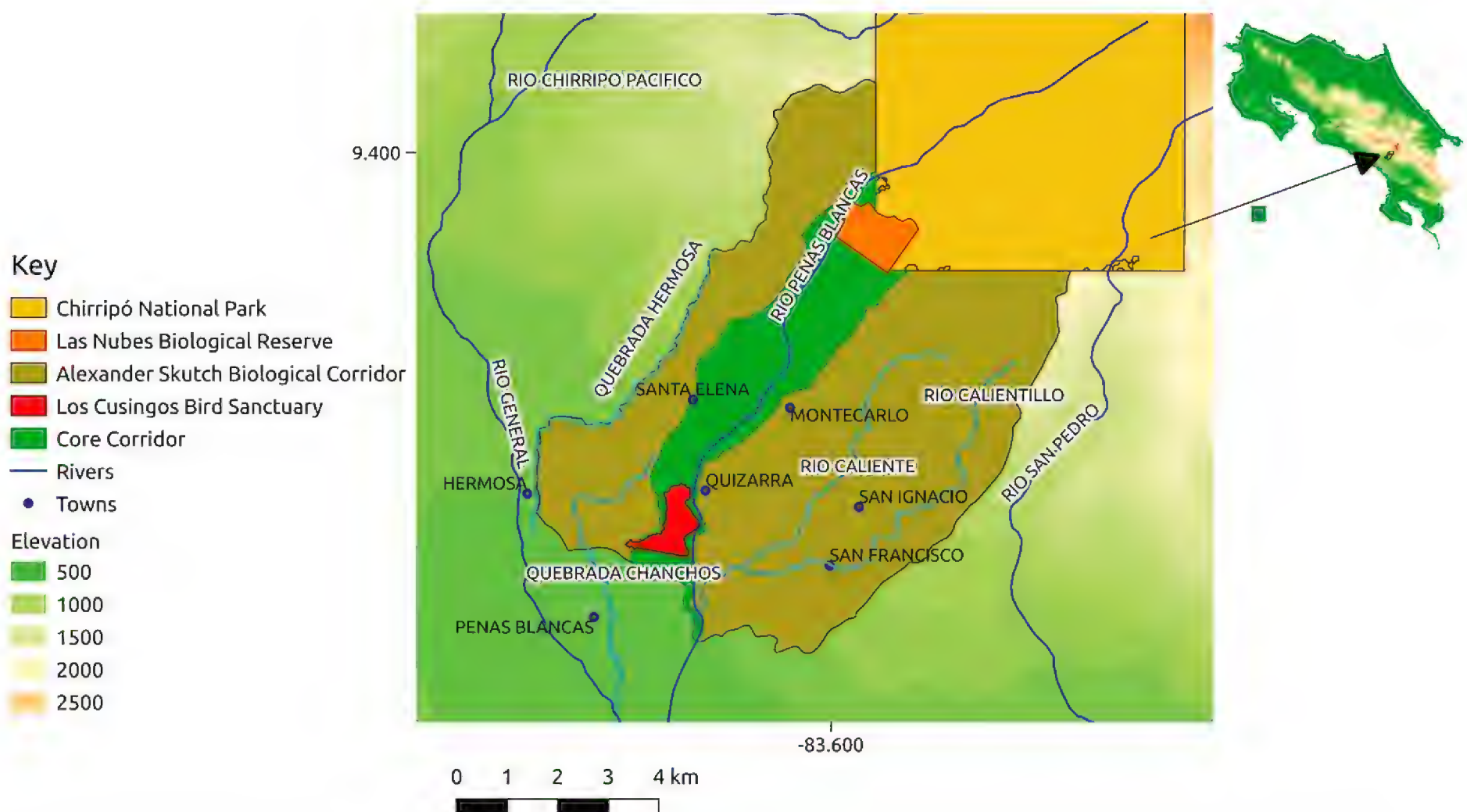


Fig. 1 Location and boundaries of the Alexander Skutch Biological Corridor (ASBC) in the province of San José, Costa Rica.

ivers. The rivers are at least 2.3 km apart in a straight line and separated by mountainous formations. Some observers had seen toads along the same river, but the sightings were 300 m apart. In-depth interviews were conducted with the observers of the Harlequin Toad incidental sightings to locate the population. After the interviews, 18 survey days followed, during which over 65 hrs were spent locating, observing, and sampling *A. varius* in the previously identified locations.

The interviews aimed to determine as accurately as possible the location, date, time, available GPS data (geo-tracking in mobile devices), weather, and relevant information of each sighting. In April and June 2016, two visits to the narrowed-down sites were conducted and systematic searches were performed. These included six independent diurnal explorations ranging from 3–5 h each, mostly starting at 7:00 AM, with night surveys also conducted to try to find individuals sleeping in the foliage. These visits were not successful in locating any individuals of the population. However, in February 2017, another search successfully located the area where *A. varius* resides. After the first individual was encountered, a transect of 1.1 km was established to monitor the identified locality systematically.

The transect was surveyed continuously for six days in February 2017, nine days in June 2017, and three days in January 2018. These months were selected to account for seasonal variation, in order to increase the chances of an encounter. Systematic surveys started early in the morning and lasted for an average of four h, with additional surveys carried out sporadically at night to identify additional locations and sites. The transect was hiked starting on opposite ends alternatively to account for possible time variations of activity of the toads. The search process usually involved 4–6 h of daily effort, walking along the river and searching in caves, rocks, foliage, and

vegetation above the river. Upon encountering the toads, they were swabbed for *Bd* samples, and individuals were measured and photographed both dorsally and ventrally. Upon the conclusion of these procedures, individuals were released in the same location, and each was handled with a separate set of gloves. Fourteen of these individuals were sampled for *Bd*, 10 during the February 2017 surveys and four during June 2017, two of which were juveniles.

Sex and age were also recorded, along with substrate, activity (Rest/sleep, Hide, Bask/Splash, Walk/Climb/Feed), and distance from the river upon first encounter. For simplicity, the behavior labelled as “splash” refers to moisture control, as toads absorb water from wet surfaces in the stream's splash zone (Pounds and Crump 1994). For better data analysis, the substrate Vegetation was classified as either trees, bromeliads, branches, or tree trunks; Soil/Rock refers to either bare ground, crevices, boulders, or mossy regions on top of the bare rock. The distance from the river was recorded with horizontal and vertical distances measured from the water's edge to the perch where the toad was found.

The skin swab and storage protocol by Whitfield et al. (2017) was used, which involves rubbing a sterilized swab (MW-100 cotton-tipped swab) on the dorsum, the venter, the sides, and the limbs. Nucleic acids were extracted from the swabs using Prepman Ultra and Real-Time PCR protocols (Boyle et al. 2004; Kriger et al. 2006) in the Laboratory of Experimental and Comparative Pathology, School of Biology, University of Costa Rica. Positive and negative controls were run in triplicate on each 96-well PCR plate. *Bd* primers and probes (Boyle et al. 2004) were used in a TaqMan® Gene Expression Assay (Applied Biosystems, Carlsbad, California). Samples were run in an Applied BioSystems Prism 7500 Sequence Detection System in Centro de Investigación

en Biología Celular y Molecular (CIBCM), University of Costa Rica. Samples corresponding to 14 animals from the study site are described.

Results

Open-ended Interviews of Incidental Sightings

In 2015, two independent incidental sightings of the Harlequin Toad were documented, photographed, and reported by local community members of the ASBC to York University's LNP Director (FM). In March 2016, a York University student spotted and photographed another *A. varius* specimen in the ASBC. Subsequently, in August 2016, new sightings of the Harlequin Toad were further documented by students and community members. Additional sightings of individuals were also reported by the Tropical Science Center Los Cusingsos Administrator Mario Mejía for May, August, and September of 2016. During these two years of incidental sightings, the authors engaged in open-ended interviews with the incidental observers to expand on their information. These interviews provided precious data for reducing the search area and narrowing the times of the day for targeted searches.

Transect Observations

In February 2017, 13 individuals in a rocky stream within the identified locations in the ASBC were photographed and identifying body patterns were noted. Eleven were males and two were females—no juveniles, eggs, or tadpoles were observed. In June 2017, six new individuals, identified by their unique body patterns, were recorded in the same study area—three females and three juveniles. In August 2017, at least one other individual was observed. In total, with the transect observations and the photographed incidental sightings, at least 25 different individuals have been identified in these locations. The sites where this population resides are along a wide, open, and fast flowing river, with a young riparian forest and some old tall trees, surrounded by a disturbed landscape, with only 10 to 20 m of riparian forest habitat in some areas.

Males had an average snout-vent length of 25.7 mm and females 34.3 mm. Male dorsal patterns appear to include more yellow and green tones, and females have a stronger orange and red coloration (see Fig. 2). This coloration is consistent with the remarks of Savage (1972) on *Atelopus* populations of Panama and Costa Rica. Juveniles, on the other hand, have no red and tend towards lime green, with patterns that are much more speckled (Fig. 3C,E). All individuals appeared to be in good health, and no external lesions were present; in most cases, the skin was colorful, vibrant, and healthy. Interestingly, the females had very wrinkly cloaca, probably due to oviposition; no males exhibited any similar skin condition. One female was molting (Fig. 2E), from whom skin samples were collected and observed under the microscope, which detected no presence of *Bd*. In total, 14 individuals were tested for *Bd*, all of which tested negative for *Bd* diagnosis.

Just one-quarter of the identified adults were females, and males were sometimes observed in groups. There was no indication of reproduction in February, while in June, juveniles were located, all of them at least 2 m above the river and in the foliage. In contrast to other populations of *A. varius*, the toads in the ASBC were mostly located above the riverbed, often found in the foliage, tree trunks, and bromeliads between 1–6 m above the water, both during day and night. Of the 37 recorded observations, only 11 individuals were seen close to the river or the splash area, and 10 of them were seen in vegetation between 3–6 m high.

Natural History Notes

During the surveys, there was ample opportunity to observe the natural history of this species, and these observations can help other scientists better locate and identify new sites in the field, especially when there are previously existing reports from community members. Despite the Harlequin Toad's bright coloration, this species can be challenging to locate in the field. For example, during our systematic exploration, the first individuals were found after almost 25 h of searching in the same location where it was later documented.

The start of diurnal activities varied among the seasons. During the dry season, toads were easily located very early in the morning; but during the wet season, toads were less frequent, and surveys needed to start much later when the day had warmed up. Nineteen sightings were obtained during January and February, but only six individuals in June, even though more time and effort was spent during this month. Overall, the sightings of females were less abundant, and for both sexes, there were more individuals seen during the dry season. There was a change in sex ratio between the two seasons (Fig. 4, $G = 4.216$, 1 df, $P = 0.040$), with proportionately more males detected during the dry season than the wet season.

Regarding the substrate, females were generally absent from the leaf litter and mostly found on the vegetation and river boulders, while males were found on the leaf litter (Fig. 5, $G = 9.035$, 2 df, $P = 0.011$). Previous records suggest that this toad is most often found along the banks, sitting on the rocks near the splash zone (Crump and Pounds 1985; Pounds and Crump 1994; Savage 2002). However, in this case, adults were typically found in the vegetation or on the ground, basking on the rocks or foraging along the river bank, and rarely seen in the splash zone (Fig. 6). Juveniles had a stronger preference for vegetation (Fig. 6, $G = 8.365$, 2 df, $P = 0.015$), which explains why they were so difficult to locate (Fig. 3E).

The toads were observed basking more during the wet season, on the rocks near the openings in the canopy or the exposed vegetation (Fig. 2E-G). For example, the three females in Fig. 2 are pictured as found: the first was basking, exposed on the vegetation in an open part of the river around 9:00 AM during the dry season; the second and third females were basking on the river rocks during the wet season. Hiding and basking were activities found to be significantly associated with the dry season, while hiding was never seen during the wet season (Fig. 7, G

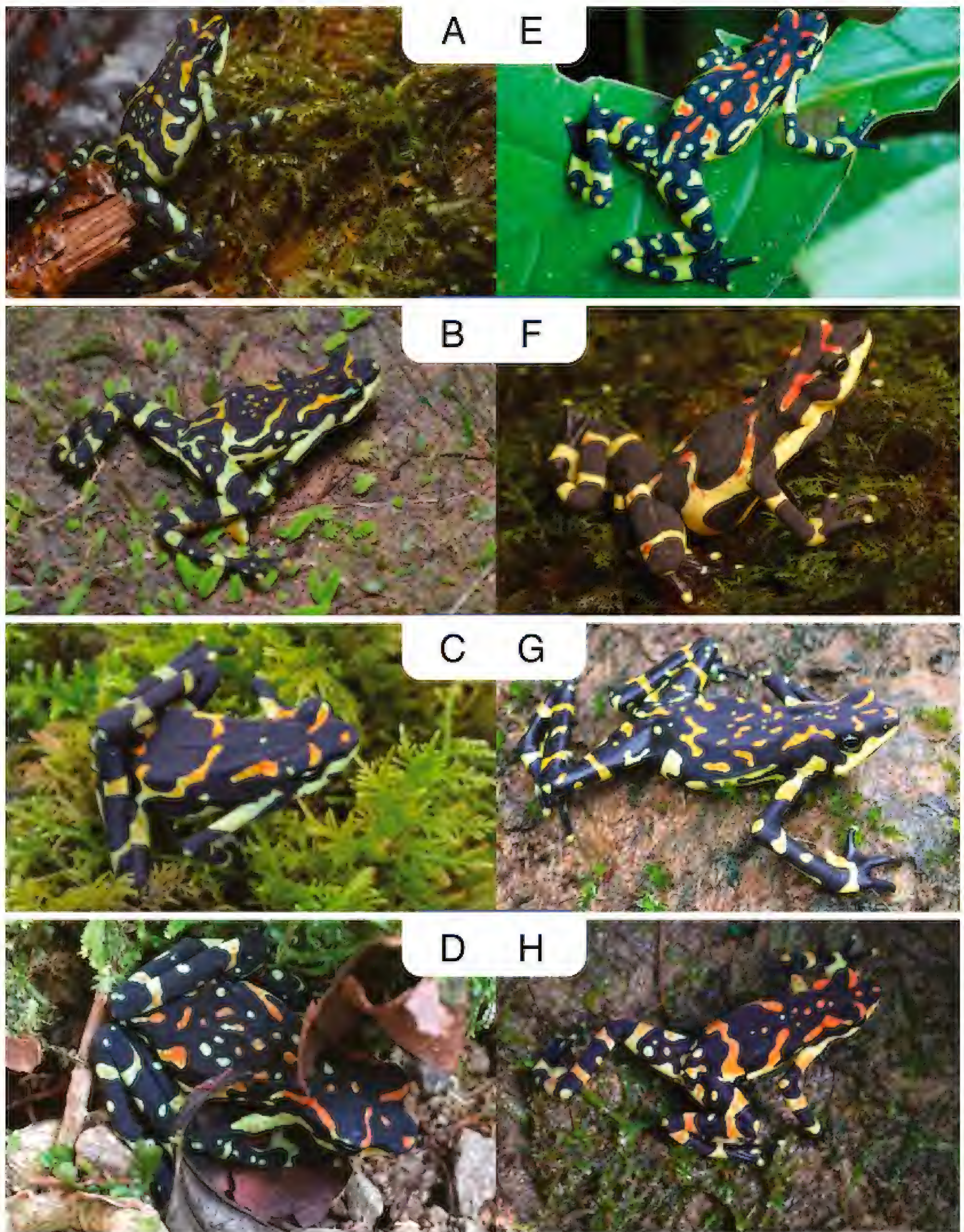


Fig. 2 Detail of *Atelopus varius* individuals found during February and June 2017 in the ASBC. The left column includes males; the right column includes females. Males (**B**) and (**C**) were photographed as found, as were females (**E**), (**G**), and (**H**). Note the spread-out basking position of female (**G**).

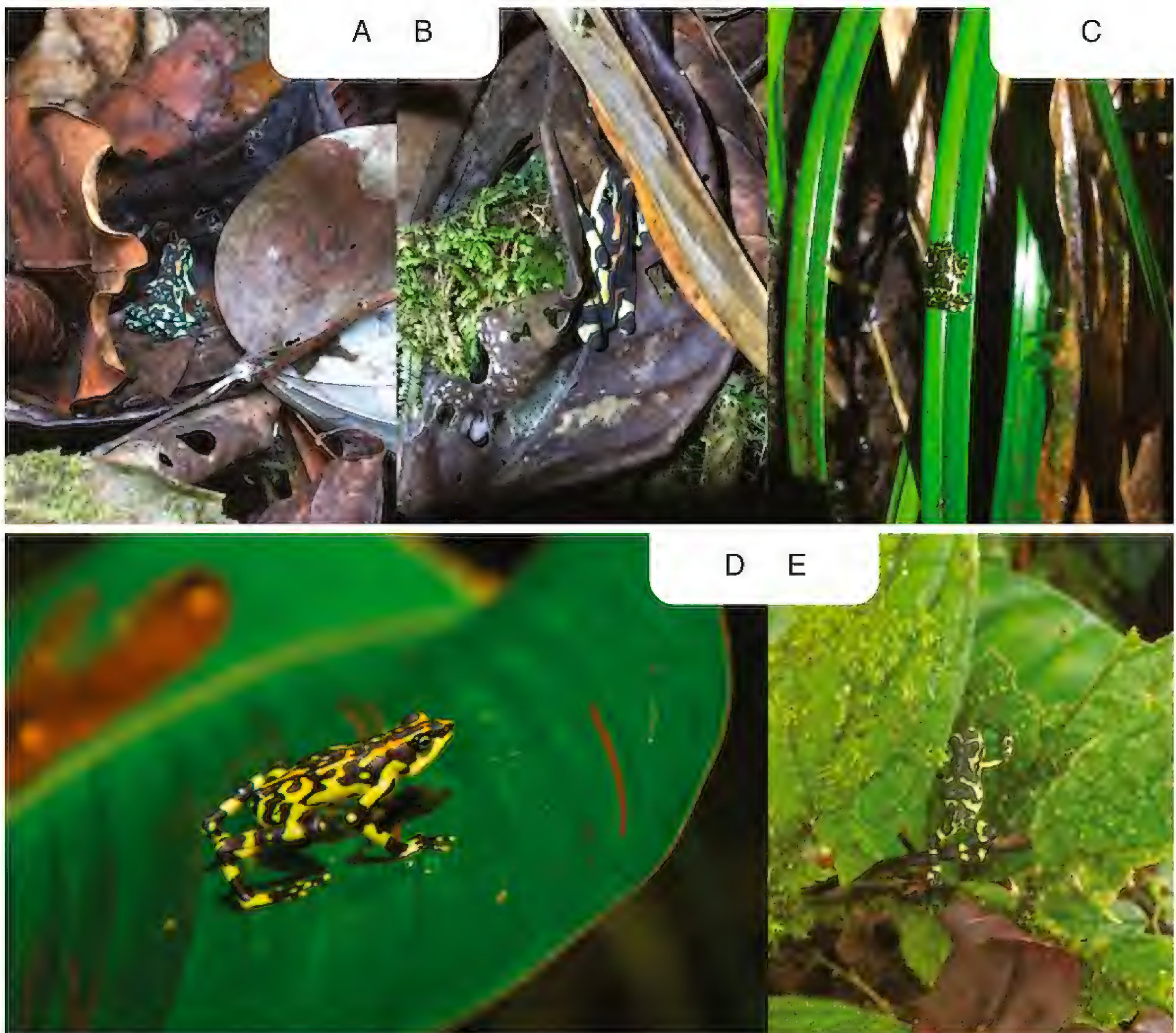


Fig. 3 Males, females, and juveniles of *Atelopus varius* found in the Alexander Skutch Biological Corridor. (A) Male found during February surveys. (B) Sleeping male in the leaf litter in the same location of a male in Fig. 2(A). (C) and (E) are juveniles high above the river bank, at least 3–4 m high in the vegetation of the understory, and juvenile (C) is sleeping. (D) A female Harlequin Toad sleeping on the vegetation five m above the river.

= 13.159, 1 df, $P = 0.004$). When rains were abundant, the toads were found sleeping or resting high on the vegetation.

A difference in activity patterns was also observed between males and females of this species (Fig. 8, $G = 15.247$, 1 df, $P = 0.002$). During this study, females were mostly seen basking on river boulders, or on top of vegetation above the river; while males were more passive, with significantly predominant activity in the leaf litter and crevices of the riverbed, hiding, or resting (Fig. 3A-B). On the other hand, the activity for juveniles of this species mostly involved sleeping or active movement for feeding. Juveniles were never observed basking or hiding like the adults (Fig. 9, $G = 9.934$, 1 df, $P = 0.019$).

The Harlequin Toads studied here seemed to be very faithful to their sleeping grounds. On five separate occasions, three individuals, including a juvenile (Fig. 3C), were seen sleeping on the same leaf during the night. Besides the observed nocturnal feeding behavior, almost

all the individuals seen during the night were sleeping in the vegetation high above the river. Activity started before dawn. One of these individuals was monitored during the night, and its activity started before 4:30 AM. Toads sleeping high in the vegetation (Fig. 3 C,D) made their way down to lower levels of the ground as the morning warmed up, with some of them remaining on the vegetation during the day (Fig. 2E).

Discussion

Based on Savage's (1972; 2002) reviews of *A. varius* distribution in Costa Rica and Panama, it is clear that most of the recently re-discovered populations have been found in areas of their historical distribution. The report by Ryan et al. (2005) for Fila Chonta, 10 km northwest of Quepos, matches the premontane distribution suggested for southwestern Costa Rica, where the nearest site identified by Savage (1972) is Barú. The account by Barrio-Amorós and Abarca (2016) for the Uvita region

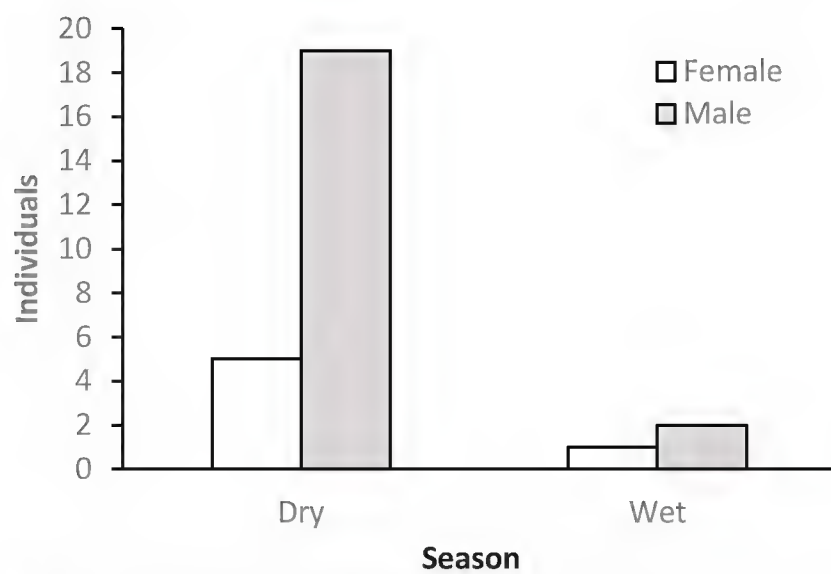


Fig. 4 Occurrence of males and females of *Atelopus varius* according to season.

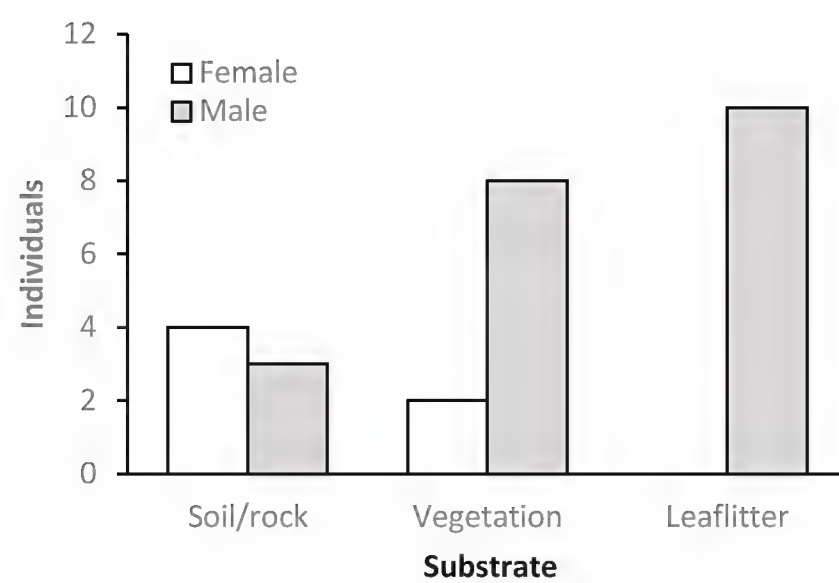


Fig. 5 Substrate preference of *Atelopus varius* according to sex.

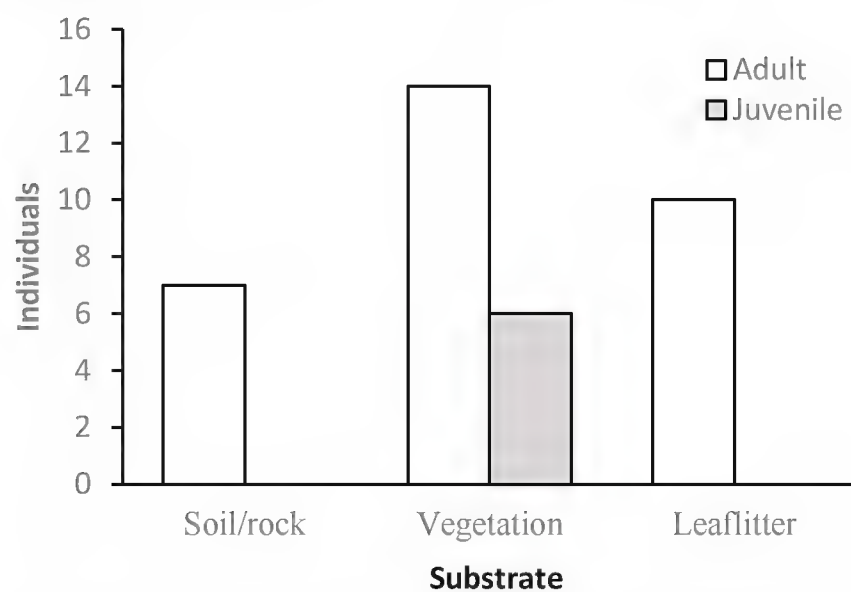


Fig. 6 Substrate preference of *Atelopus varius* according to age.

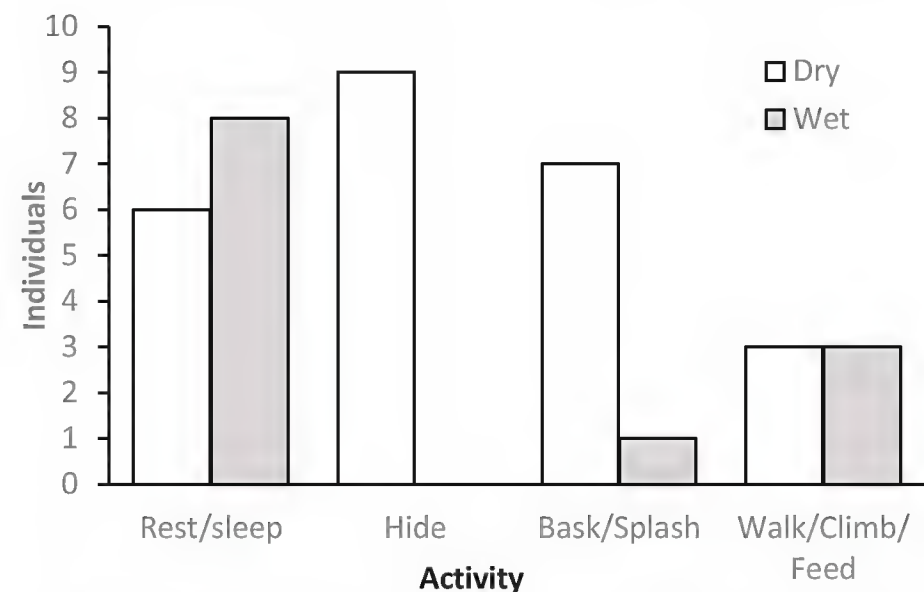


Fig. 7 Activity pattern of *Atelopus varius* according to season.

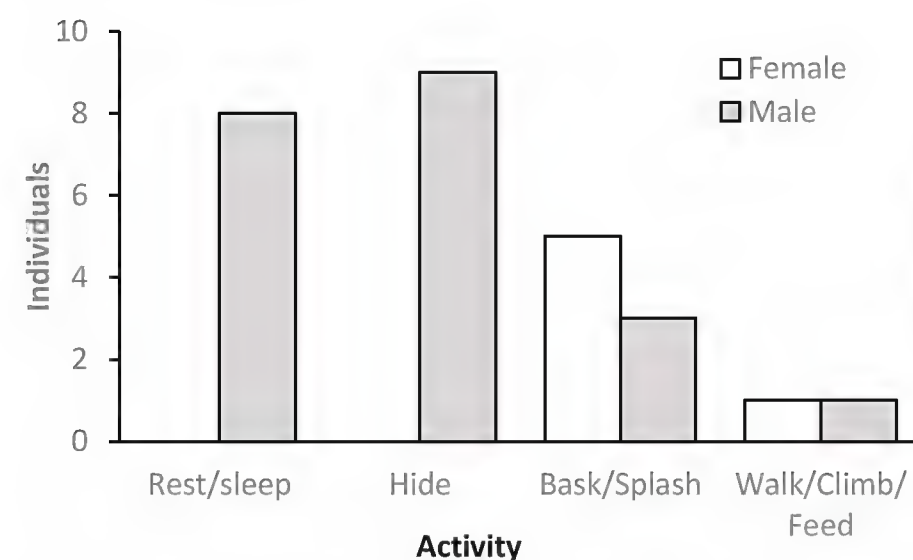


Fig. 8 Activity pattern of *Atelopus varius* according to sex.

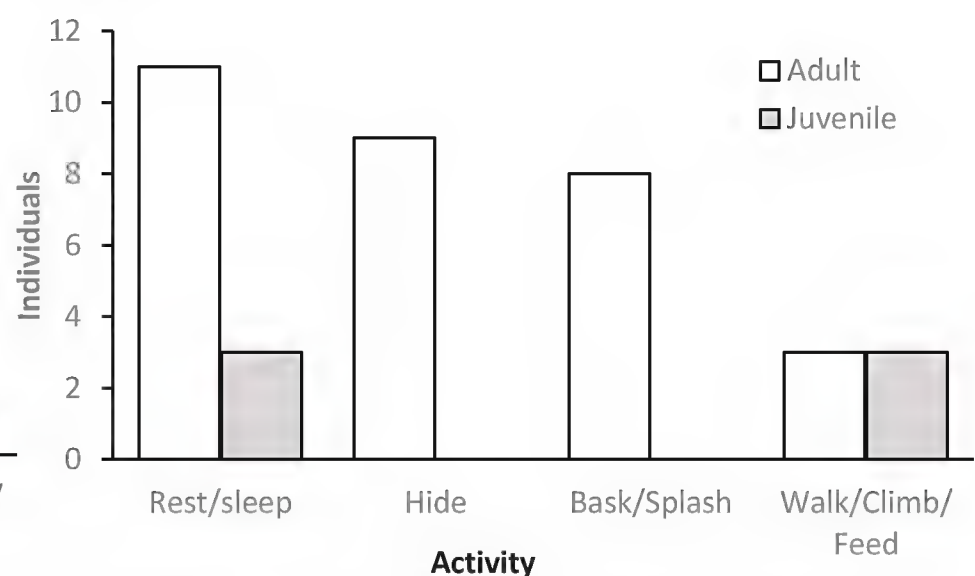


Fig. 9 Activity pattern of *Atelopus varius* according to age.

matches with that reported by Savage (1972); and finally, the rediscovery in Las Tablas by González-Maya et al. (2013), brings hope for the survival of the toads near Cotón, also previously identified by Savage (1972). These findings confirm that some populations in the Pacific versant have managed to escape the decline while persisting in the same localities, at least so far. However, evidence from our research, with the discovery of a new locality, offers augmented hope for species recovery. We hypothesize that the behavior of this population could have helped to reduce the risk of exposure to *Bd* infection and also to allow a possible change in the historical distribution of this species.

These results, and the similarity between both the scientific and community accounts discussed below, suggest that *A. varius* distribution could have

changed to penetrate higher in the montane regions of the Talamanca Mountain Range in the Pacific Slope. Despite the lack of specimens for the area and the lack of a collection archive, previous evidence has recorded *A. varius*, specifically this ecomorph, in the San Isidro del General region at 704 m asl (Savage 1972), with no other accounts found by the authors for the ASBC area (from 1,100–1,500 m asl). Furthermore, Savage (1972) refers to this toad as a predominantly premontane frog with penetration into lower montane zones only at six localities in Costa Rica, none of which includes the area of this study—the closest area identified is Pérez Zeledón at 704 m asl (Savage 1972). Taking Savage's (1972) account into consideration, the discovery of this population in montane areas of the ASBC suggests this is an undocumented locality. This scientific account is

congruent with interviews of highly experienced locals who do not report or remember this species being present at elevations higher than 600–700 m asl.

Long-time residents of what is now the Alexander Skutch Biological Corridor remembered that the Harlequin Toad had been abundant in the area some 50 years ago, but that this toad had not been reported again for decades, until now. During initial interviews, local observers showed good competency to immediately identify Harlequin Toads. One of them acknowledged the remarkable altitudinal change for this species over the years, “*this frog was very common at 600 m over twenty years ago, now it is over 1,000 m. It was never here, and it has come higher in the mountain. It’s the first time I see it again in all these years*” (Ramón Mora). This citizen testimony along with Savage’s (1972) account could point to a possible geographic displacement. When considering the possible displacement, we acknowledge that reduced biodiversity exploration in the area could explain how this population went under the scientific radar for so long; yet, with no valid reason, this assumption would disregard the confidence, skills, experience, and traditional knowledge of the inhabitants that are quite confident the frog was not present over 700 m asl in the past. If this species were not so easily identified, with such a reduced risk of misidentification in the field, we would consider this possibility. This possible change in distribution might be helping to facilitate its continuing survival through some environmental stressors, such as climate change and habitat loss. It might also increase the risk of *Bd* exposure, and clearly more research is needed to corroborate this possible explanation, exploring the trade-offs between adaptation to environmental changes and *Bd* exposure.

The reappearance of *A. varius* in this region could be the result of several possible factors. One hypothesis is that establishing the biological corridor in 2005, and subsequent efforts to enhance ecological connectivity between forest patches, have allowed for the recovery of habitats that previously served as niches for relict populations on the verge of extinction, allowing those populations to recover and relocate, possibly pushed by environmental changes. Another potential explanation has to do with the possible emergence of resistance among these threatened relict populations of *A. varius* to the previously devastating *Bd* fungal disease (Perez et al. 2014).

To expand on the emergence of resistance to the *Bd* infection hypothesis, we present two critical remarks that contribute to the natural history of the species and might be related to this population’s survival. First, the toads in the area are seen less often on the splash region of the river, and more often on the vegetation, regardless of seasonality. Second, the typical sedentary behavior in the mossy rocks near the splash zone associated with this species (Pounds and Crump 1994) is not as typical in this location. The toads are not so sedentary, and basking seems to be more important here than capturing moisture in the splash zone. Many authors, including Pounds and Crump (1994), recognize that this species depends on the moisture of the splash zone and individuals tend to aggregate in the waterfall splash with the progression

of the dry season. The experiences of working with this population complement this information. Here, toads are regularly seen in the foliage, tree trunks, and the leaf litter, and not on the splash zone, only seven of the 37 registered encounters were found on the river rocks or crevices. The basking behavior is as important, or perhaps more so, than moisture-seeking behavior. The toads appear to move to open areas on the river bank or the exposed vegetation where there is more solar radiation; and the selection of such areas was more frequent during the warmest time of the morning, especially on colder days. This selection could also explain the difference of activity during dry and wet seasons, where during the wet season the toads were “lazier” in the early hours of the morning when it was cold and they were found closer to the river when the day was warmest. These individuals were much less sedentary than previously reported, engaging in significant daily movements. Anurans sleeping on the vegetation 3–4 m above the river move during the morning down to the river bank; explaining why this species has been hard to locate in the area, as researchers might focus solely on crevices, rocks, and roots as reported by most of the literature. Understanding this was the key to finding the juveniles that were far from the river and much higher than eye-level.

In other words, the natural history remarks noted above suggest that *A. varius* spends less time closer to the river and more time in open areas basking; which in turn might be linked to available solar radiation and moisture control. Temperature (Woodhams et al. 2003) and moisture (Johnson et al. 2003) have been suggested as two important environmental factors influencing the growth and survival of *B. dendrobatidis*. So this behavior could explain why this toad survived in an area cataloged as highly likely for the existence of *Bd* (Puschendorf et al. 2009). Furthermore, the negative results of the *Bd* test support this theory. The documented absence of infection in these Harlequin Toads, a highly susceptible species in an area identified with a high probability for occurrence of *Bd*, allows us to hypothesize that spending less time closer to the river and more time in open areas basking, could help the toads of this population survive by reducing infection risk. The findings of Woodhams and colleagues (2003) support our hypothesis; they present evidence that short periods of high body temperature can eliminate the pathogen from its hosts, with experiments suggesting that normal thermoregulation can clear frogs of chytrid infection (Woodhams et al. 2003). We hope this account can help provide a better understanding of the behavior of *Atelopus*, as this behavior might hold clues for the frog’s resistance to *Bd* or climatic changes, and can also help to locate new populations.

Conclusions

García-Rodríguez et al. (2012) ask “*Where are the survivors [of] relict populations of endangered frogs in Costa Rica?*” In this research, both community members and scientists have contributed to answering this question. Moreover, with *A. varius* being among the most endangered of all amphibian species (La Marca et al. 2005), it is certain that the discovery of the Harlequin

Toad in the Alexander Skutch Biological Corridor makes the conservation efforts in this biological corridor of critical importance. This population is particularly relevant because of ongoing attempts by private companies to obtain permits for building a hydroelectric plant that would dam the two major streams in the ASBC, destroying the habitats successfully recovered during a decade of conservation efforts in the corridor. The reappearance of the Harlequin Toad, currently classified as Critically Endangered (IUCN 2015), lends supporting evidence to the effectiveness of this habitat recovery.

The active involvement of citizens in the discovery of *A. varius* in the ASBC points to the importance of strengthening the citizen science component to further the knowledge base regarding this and other species in the area. The historical role of citizen science in ecological research has been generally overlooked, despite its significant contributions (Miller-Rushing et al. 2012; Dickinson et al. 2012). The opportunistic and widespread nature of these sightings suggests that strengthening citizen science approaches can help to maximize resources and opportunities for encountering this and other rare, endangered species. Citizen science creates a nexus between science and education that expands the frontiers of ecological research and public engagement (Newman et al. 2012). After all, without the enthusiastic and curiosity-filled reports from the community, the re-emergence of this rare and endangered species might have gone completely undetected by the scientific community. Moreover, this participatory process can generate new meanings and values for herpetofauna in the community, leading to improved human behaviors directed at the protection of these and other vulnerable species.

In addition to continuing to monitor for the presence of *A. varius* in the ASBC, the community initiative that has already documented the reappearance of the Harlequin Toad in the biological corridor will also be useful in providing further data to help answer pending questions regarding the impacts of climate change, fungal disease, and pesticides on these fragile populations.

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Literature Cited

- Barrio-Amorós C, Abarca J. 2016. Another surviving population of the critically endangered *Atelopus varius* (Anura: Bufonidae) in Costa Rica. *Mesoamerican Herpetology* 3: 128–134.
- Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocumbe R, Ragan MA, Hyatt AD, McDonald KR, et al. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences of the United States of America* 95(15): 9,031–9,036.
- Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* 60: 141–148.
- Crump ML, Pounds JA. 1985. Lethal parasitism of an aposematic anuran (*Atelopus varius*) by *Notochaeta bufonivora* (Diptera: Sarcophagidae). *Journal of Parasitology* 71(5): 588–591.
- Dickinson JL, Shirk J, Bonter D, Bonney R, Crain RL, Martin J, Phillips T, Purcell K. 2012. The current state of citizen science as a tool for ecological research and public engagement. *Frontiers in Ecology and the Environment* 10(6): 291–297.
- García-Rodríguez A, Chaves G, Benavides-Varela C, Puschendorf P. 2012. Where are the survivors? Tracking relictual populations of endangered frogs in Costa Rica. *Diversity and Distribution* 18(2): 204–212.
- González-Maya JF, Belant JL, Wyatt SA, Schipper J, Cardenal J, Corrales D, Cruz-Lizano I, Hoepker A, Escobedo-Galván AH, Castañeda F, et al. 2013. Renewing hope: the rediscovery of *Atelopus varius* in Costa Rica. *Amphibia-Reptilia* 34(4): 573–578.
- IUCN. 2012. Annex 6 “Rules of procedure IUCN Red List assessment process 2013–2016”. IUCN SSC Steering Committee. Available: https://cmsdata.iucn.org/downloads/rules_of_procedure_for_red_list_2013_2016_final.pdf. [Accessed: 1 January 2018].
- IUCN. 2015. The IUCN Red List of threatened species. Version 2015–4. Available: <https://www.iucnredlist.org>. [Accessed: 8 February 2017].
- Johnson ML, Berger L, Philips L, Speare R. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 57(3): 255–260.
- Kruger KM, Hines HB, Hyatt AD, Boyle DG, Hero J-M. 2006. Techniques for detecting chytridiomycosis in wild frogs: comparing histology with real-time Taqman PCR. *Diseases of Aquatic Organisms* 71(2): 141–148.
- La Marca E, Lips KR, Lötters S, Puschendorf R, Ibáñez R, Rueda-Almonacid JV, Schulte R, Marty C, Castro F, Manzanilla-Puppo J, et al. 2005. Catastrophic population declines and extinctions in neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica* 37(2): 190–201.
- Miller-Rushing A, Primack R, Bonney R. 2012. The history of public participation in ecological research. *Frontiers in Ecology and the Environment* 10(6): 285–290.

- Newman G, Wiggins A, Crall A, Graham E, Newman S, Crowston K. 2012. The future of citizen science: emerging technologies and shifting paradigms. *Frontiers in Ecology and the Environment* 10(6): 298–304.
- Perez R, Richards-Zawacki CL, Krohn AR, Robak M, Griffith EJ, Ross H, Gratwicke B, Ibáñez R, Voyles J. 2014. Field surveys in Western Panama indicate populations of *Atelopus varius* frogs are persisting in regions where *Batrachochytrium dendrobatidis* is now enzootic. *Amphibian & Reptile Conservation* 8(2): 30–35 (e85).
- Pounds JA, Crump ML. 1994. Amphibian declines and climate disturbance: the case of the Golden Toad and the Harlequin Frog. *Conservation Biology* 8(1): 72–85.
- Pounds J, Puschendorf R, Bolaños F, Chaves G, Crump M, Solís F, Ibáñez R, Savage JM, Jaramillo C, Fuenmayor Q, et al. 2010. *Atelopus varius*. The IUCN Red List of threatened species. Available: <https://www.iucnredlist.org/details/54560/0> [Accessed: 19 December 2017].
- Puschendorf R, Carnaval A, VanDerWal J, Zumbado-Ulate H, Chaves G, Bolaños F, Alford RA. 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Diversity and Distribution* 15: 401–408.
- Ryan M, Berlin E, Gagliardo R. 2005. Further exploration in search of *Atelopus varius* in Costa Rica. Available: <http://www.amphibians.org/froglog/fl069/> [Accessed: 3 March 2018].
- Savage JM. 1972. The Harlequin Frogs, genus *Atelopus*, of Costa Rica and western Panama. *Herpetologica* 28(2): 77–94.
- Savage JM. 2002. *The Amphibians and Reptiles of Costa Rica: A Herpetofauna between Two Continents, between Two Seas*. University of Chicago Press, Chicago, Illinois, USA. 934 p.
- Solano Cascante JC, Solano Cascante BJ, Boza Oviedo EE, Vargas Quesada J, Sandí Méndez D. 2014. Nota Informativa: Hallazgo del sapo payaso *Atelopus varius* (Anura: Bufonidae) en La Luchita, Buenos Aires, Puntarenas, Costa Rica. Proyecto Biodiversidad de Costa Rica.
- Whitfield SM, Alvarado G, Abarca J, Zumbado H, Zuñiga I, Wainwright M, Kerby J. 2017. Differential patterns of *Batrachochytrium dendrobatidis* infection in relict amphibian populations following severe disease-associated declines. *Diseases of Aquatic Organisms* 126(1): 33–41.
- Woodhams DC, Alford RA, Marantelli G. 2003. Emerging disease of amphibians cured by elevated body temperature. *Diseases of Aquatic Organisms* 55(1): 65–67.
- Woodhams DC, Alford RA, Briggs CJ, Johnson M, Rollins-Smith LA. 2008. Life-history trade-offs influence disease in changing climates: strategies of an amphibian pathogen. *Ecology* 89(6): 1,627–1,639.



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Thorius narismagnus (Amphibia: Plethodontidae): rediscovery at the type locality and detection of a new population

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Abstract.—Of the 42 Critically Endangered species of plethodontid salamanders that occur in Mexico, thirteen have not been reported in more than ten years. Given the lack of reports since 1976, the minute plethodontid salamander *Thorius narismagnus* is widely considered as missing. However, this report describes the rediscovery of this minute salamander at the type locality (Volcán San Martín), as well as a new locality on Volcán Santa Marta, 28 km southeast of its previously known distribution, both in the Los Tuxtlas region of Veracruz, Mexico. The localities where *T. narismagnus* has been found are mature forests in a community reserve on Volcán San Martín and a private reserve on Volcán Santa Marta. The presence of maxillary teeth, generally absent in *Thorius*, are reported here in some *T. narismagnus* females. Two efforts which may contribute to the conservation of *Thorius narismagnus* are the preservation of the cloud forests where this species persists, as well as the determination of the presence and possible effect of chytrid fungus in these populations.

Keywords. Conservation, ecological reserve, Los Tuxtlas, missing species, minute salamander, molecular analysis, Mexico

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Introduction

In Mexico, 132 species of plethodontid salamanders have been recorded (AmphibiaWeb 2019), 42 of which are Critically Endangered (CR) according to the International Union for the Conservation of Nature (IUCN 2019). This conservation status may be assigned due to various combinations of biological characteristics (e.g., restricted distribution or specific environmental requirements) and risk factors (e.g., habitat modification or climate change; Stuart et al. 2008). In recent decades, population declines have been observed or estimated for several species (Frias-Alvarez et al. 2010) and in some cases, despite intensive search efforts, finding them has not been possible.

According to the IUCN (2019), 13 Critically Endangered plethodontid species in Mexico have not been reported in more than 10 years. Since 2010, six of these species have been rediscovered: *Isthmura naucampatepetl* (Naturalista 2019), *Chiropterotriton magnipes*, *C. mosaueri*, *Pseudoeurycea ahuitzotl*, *P. tlahcuiloh*, and

Thorius munificus (AmphibiaWeb 2019). However, so far there have been no subsequent records of the remaining seven species, including *Thorius narismagnus*. The unknown status of these species highlights the need to carry out sampling efforts in their historical localities and to explore those areas with favorable environmental conditions, in order to locate new populations (Sandoval-Comte et al. 2012).

Thorius narismagnus (Shannon and Werler 1955) is a minute salamander with a known distribution that comprises only four localities, not more than 8 km apart, on Volcán San Martín in the Los Tuxtlas region, Veracruz, Mexico (Fig. 1), in an elevational range between 890 and 1,200 m asl (Hanken and Wake 1998). *Thorius narismagnus* is considered as CR because the extent of its occurrence is < 17 km² with reductions in the extent and quality of its habitat, and due to a continuing decline in the number of mature individuals (IUCN 2016). The historical records of *T. narismagnus* include 55 specimens collected between 1953 and 1976, and since

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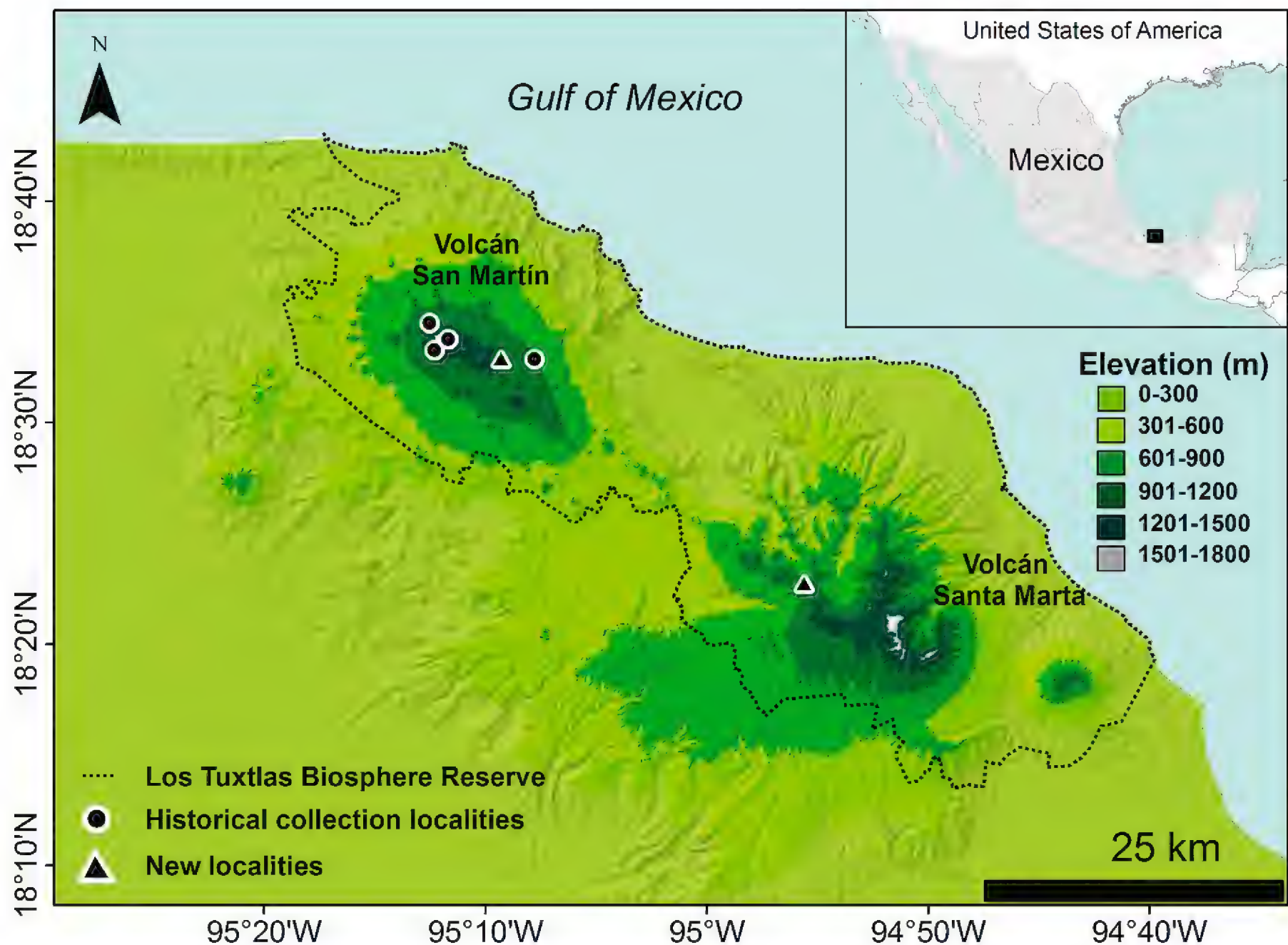


Fig. 1. Locations of historical collection localities and the new localities of *Thorius narismagnus* in Los Tuxtlas region, Mexico.

then this species has not been reported (IUCN 2016).

Rediscovery

As part of a study on the diversity and conservation of amphibians in Veracruz, fieldwork was carried out in a cloud forest in the community reserve Ejido Ruiz Cortines, San Andrés Tuxtla, Veracruz (18°32'53"N, 95°09'16"W; 1,136 m asl) on Volcán San Martín (Fig. 1; Fig. 2A) and in the private Ecological Reserve "La Otra Opción" Catemaco, Veracruz (18°22'32"N, 94°55'28"W; 1,075 m asl) on Volcán Santa Marta (Fig. 1; Fig. 2B). At both sites searches for amphibians (08:00–12:00 and 20:00–00:00 h) were conducted in all terrestrial microhabitats commonly used by these organisms (Crump and Scott 1994).

In the Ejido Ruiz Cortines community reserve, with a cumulative search effort of 72 person-hours in September 2012, three individuals of the genus *Thorius* were detected, two of which were collected (from which a tissue sample was taken and the individuals were subsequently preserved) and deposited in the Colección de Anfibios y Reptiles del Instituto de Ecología A.C. (CARIE 0857, 1137; Fig. 2C). A sampling effort of 24 person-hours was carried out in July 2015 at this locality, but there were no sightings of any minute salamanders. The two collected specimens measured 17.6 and 11.4

mm in SVL, the ratios between the length and width of the nostrils were 1.14 and 1.09, with four and five free intercostal grooves separating the adpressed fore and hind limbs, respectively.

In the private ecological reserve La Otra Opción, with cumulative search efforts of 96 person-hours in July 2015 and 66 person-hours in July 2017, three individuals of *Thorius* were detected in well-preserved forests in each year. The three individuals detected in 2015 were captured (each one was measured and a sample of tail tissue was taken) and subsequently released; these specimens had SVL measurements of 15.4, 15.9, and 19.4 mm. The three individuals found in 2017 were collected (CARIE 1251, 1258, 1259; Fig. 2D) and a sample of tissue was taken from each; they measured 19.9, 22.5, and 21.2 mm SVL, respectively. The proportions between the length and width of the nostrils were 1, 1, and 1.15, respectively, with 5.5 free intercostal grooves separating adpressed limbs. Two females from La Otra Opción (CARIE 1251 and 1259) had eight and five maxillary teeth, respectively, while the adult male (CARIE 1258) lacked maxillary teeth.

Identification of Specimens

The coloration in life of the specimens collected in the Volcán San Martín and Volcán Santa Marta localities



Fig. 2. Habitat in the Volcán San Martín locality (A) and a specimen (C) collected from it in life (CARIE 0857). Habitat in the Volcán Santa Marta locality (B) and a specimen (D) collected from it in life (CARIE 1251).

was light brown with a dark brown spike pattern in the dorsum, dark brown color on the sides and a dark venter with small white spots (Fig. 2C, 2D). With the exception of the presence of maxillary teeth in two females, the morphological characters and the coloration coincide with the diagnosis proposed by Shannon and Werler (1955) and Rovito et al. (2013) for *Thorius narismagnus*. Although maxillary teeth are absent in most species of *Thorius*, they are present in several species, including *T. smithi*, which is relatively closely related to *T. narismagnus*. Furthermore, at least two species (*T. grandis* and *T. omiltemi*) have maxillary teeth present only in females, and maxillary teeth are more common in females of *T. minydemus* than in males, which rarely have them (Hanken and Wake 1998; Hanken et al. 1999). Maxillary teeth were absent in a total of 18 specimens from the type locality of *T. narismagnus* on Volcán San Martín as reported by Shannon and Werler (1955) and Hanken and Wake (1998), suggesting that they do not occur in either sex at that locality.

In order to confirm that both populations belong to *Thorius narismagnus*, DNA was extracted from liver tissue of one specimen from Volcán San Martín (the type locality of *T. narismagnus*) and two specimens from Volcán Santa Marta using a salt extraction protocol. A

784 bp fragment of the cytochrome b gene was amplified using primers MVZ15 and MVZ16 (Moritz et al. 1992). PCR consisted of an initial denaturation step of 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 48 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. PCR products were purified using ExoSAP IT (USB Corporation, Cleveland, Ohio, USA) and sequenced using the BigDye v3.1 terminator cycle sequencing kit (Applied Biosystems, Foster City, California, USA) on an ABI 3730 capillary sequencer. Sequences were edited using Geneious v8.1.8 (BioMatters, Auckland, New Zealand), and sequences used in analysis were 750 bp long after removing low-quality bases. Sequences for other species of *Thorius* were obtained from GenBank and sequences were aligned using Muscle v.3.8 (Edgar 2004). The pairwise GTR distance between sequences from the two populations was calculated using PAUP v4.165 (Swofford 2003). Sequences are deposited in GenBank (Table 1).

The average pairwise divergence for cytochrome b (cytb) between the two populations was 1.4% (Table 1). This level of divergence is comparable to, or lower than, that seen between conspecific populations of various species of *Thorius*. Several species of *Thorius*

Table 1. Percentage of average pairwise divergence for cytochrome b between the populations of *Thorius* species in GenBank.

	Species	Voucher	GenBank accession number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	<i>T. arboreus</i>	IBH22720	KC884119	-																															
2	<i>T. aureus</i>	IBH22356	KC884065	11.2	-																														
3	<i>T. boreas</i>	IBH22339	KC884066	11.4	8.7	-																													
4	<i>T. dubitus</i>	MCZA137386	KC884068	16.6	16.0	18.7	-																												
5	<i>T. grandis</i>	MZFC27550	KC884115	10.6	11.4	12.3	16.7	-																											
6	<i>T. insperatus</i>	IBH22901	KC884120	19.1	18.2	17.2	15.3	16.9	-																										
7	<i>T. longicaudus</i>	MCZA148744	KC884096	7.9	8.5	10.0	14.8	7.3	15.3	-																									
8	<i>T. lunaris</i>	IBH22341	KC884069	18.8	19.9	18.7	18.8	20.8	20.6	19.6	-																								
9	<i>T. magdougalli</i>	IBH22890	KC884072	8.1	8.8	11.6	14.5	9.5	17.5	8.9	20.8	-																							
10	<i>T. magnipes</i>	IBH22918	KC884122	18.5	18.4	17.8	13.7	17.6	15.2	18.8	18.2	16.9	-																						
11	<i>T. maxillabrochus</i>	MCZA148743	KC884105	18.1	20.7	21.2	15.3	19.2	18.2	19.6	21.8	16.0	15.7	-																					
12	<i>T. minutissimus</i>	IBH23011	DQ640021	13.1	12.4	13.5	17.5	15.1	18.4	12.5	18.1	12.8	17.1	22.8	-																				
13	<i>T. minydemus</i>	MVZ229269	KC884100	23.3	28.4	28.7	17.5	22.9	19.1	24.7	22.2	23.0	16.3	8.4	24.0	-																			
14	<i>T. munificus</i>	IBH29716	KC884073	19.1	19.2	19.3	19.8	23.1	21.8	18.6	5.6	20.6	19.8	19.2	20.6	21.6	-																		
15	<i>T. narismagnus</i>	CARIE1251	MK761019	19.4	18.7	20.0	16.3	19.3	19.1	18.3	20.9	18.1	17.7	19.9	20.7	22.8	22.2	-																	
16	<i>T. narismagnus</i>	CARIE1258	MK761018	19.4	18.7	20.0	16.3	19.3	19.1	18.3	20.9	18.1	17.7	19.9	20.7	22.8	22.2	0.0	-																
17	<i>T. narismagnus</i>	CARIE0857	MK761017	19.8	19.4	21.2	17.2	19.5	19.4	18.2	20.9	18.8	17.6	19.8	21.7	22.2	22.1	1.4	1.4	-															
18	<i>T. narisovalis</i>	IBH26500	KC884077	12.2	10.3	11.0	18.6	14.6	15.6	9.4	19.1	11.4	17.3	19.1	12.1	22.7	19.8	18.9	18.9	18.6	-														
19	<i>T. omilemi</i>	MVZ269309	KC884079	11.2	11.2	12.6	17.5	7.3	17.9	7.3	22.9	10.2	18.0	18.3	16.5	21.9	22.1	18.6	18.6	18.5	14.1	-													
20	<i>T. papaloae</i>	IBH22355	KC884081	10.7	10.0	11.1	17.4	11.4	16.5	9.0	19.7	9.3	17.2	18.8	16.1	22.4	20.0	18.8	18.8	18.5	10.8	10.1	-												
21	<i>T. pennatulus</i>	IBH26499	KC884082	20.4	20.7	20.5	18.5	19.9	20.2	18.5	20.1	20.0	18.0	18.9	21.1	18.8	19.8	8.8	8.8	8.6	22.1	18.4	19.9	-											
22	<i>T. pinicola</i>	IBH13995	KC884094	11.9	8.5	10.9	15.8	8.3	16.8	6.0	19.4	11.5	16.9	18.9	13.6	21.9	18.9	18.5	18.5	18.4	11.3	6.3	10.9	18.5	-										
23	<i>T. pulmonaris</i>	MCZA148742	KC884101	8.8	10.6	12.5	18.8	6.6	14.8	6.5	15.5	11.4	18.9	18.9	14.4	20.4	15.0	20.1	20.1	20.1	10.7	7.2	7.9	20.3	4.6	-									
24	<i>T. schmidtii</i>	MVZ269312	KC884085	19.3	21.0	20.0	12.1	19.8	18.3	19.7	22.6	19.8	9.2	15.6	20.5	17.3	22.4	17.7	17.7	18.6	19.5	21.2	18.9	18.9	18.7	20.9	-								
25	<i>T. smithi</i>	IBH26615	KC884121	19.6	18.6	18.7	15.8	20.9	13.6	16.9	21.2	18.2	17.0	14.9	19.8	16.6	20.0	10.3	10.3	10.2	17.5	18.5	16.6	11.8	17.7	18.5	17.4	-							
26	<i>T. sp. 1</i>	GP0099	KC884086	21.1	21.6	22.7	15.6	18.7	15.2	19.4	25.1	17.8	17.7	6.0	21.2	4.5	24.1	19.3	19.3	19.1	18.6	18.2	18.0	17.6	17.9	18.2	18.0	15.4	-						
27	<i>T. sp. 2</i>	MCZA148757	KC884118	9.6	9.4	10.4	17.2	8.1	16.2	6.2	17.9	9.6	18.6	19.9	13.1	22.3	18.3	18.4	18.4	18.3	12.4	7.5	9.9	17.7	6.9	6.6	22.6	18.1	18.8	-					
28	<i>T. sp. 3</i>	MCZA148759	KC884116	9.3	7.9	12.7	14.0	8.7	16.8	5.7	22.9	8.0	16.7	22.8	11.5	31.3	24.6	18.4	18.4	18.4	10.7	7.8	8.7	17.7	6.2	9.0	17.3	19.9	15.9	7.8	-				
29	<i>T. sp. 7</i>	MCZA148754	KC884111	13.2	11.7	13.9	19.7	14.4	18.5	11.3	22.4	9.8	22.4	19.7	18.1	25.3	22.0	20.6	20.6	21.0	13.7	13.2	9.3	22.5	12.7	13.2	21.9	19.2	17.7	12.4	10.2	-			
30	<i>T. spilogaster</i>	IBH22975	KC884087	18.5	20.3	21.1	14.2	18.2	14.4	18.8	20.5	17.5	14.8	7.4	20.0	2.3	20.8	17.7	17.7	18.1	18.5	18.0	17.0	16.2	17.2	17.3	15.6	14.5	4.5	18.0	14.2	17.6	-		
31	<i>T. tlaxiacus</i>	MCZA148745	KC884098	9.8	10.6	12.0	16.0	7.3	16.0	5.0	19.5	9.0	16.2	18.6	13.7	23.2	19.0	17.7	17.7	17.7	12.5	6.5	10.3	17.3	5.9	6.8	18.8	19.0	19.0	6.6	5.1	13.3	17.3	-	
32	<i>T. troglodytes</i>	IBH222981	KC614433	18.7	19.7	19.6	12.9	17.4	17.8	16.4	20.4	16.2	13.9	16.7	19.0	17.0	20.4	15.4	15.4	15.8	18.7	16.4	18.3	16.4	16.0	19.0	14.8	14.3	16.8	17.4	17.2	20.5	14.2	16.6	-

from Oaxaca, including *T. boreas*, *T. macdougalli*, and *T. narisovalis*, have substantially higher divergence than that seen between the two populations from Los Tuxtlas that were sequenced here. For example, two populations of *Thorius boreas* separated by only 17 km are 5% divergent for cytb (Rovito et al. 2013). The close genetic similarity between the populations from Volcán Santa Marta and from the type locality of Volcán San Martín strongly suggests that these two populations are conspecific.

Conservation Implications

The record from the Ejido Ruiz Cortines community reserve locality represents the rediscovery of *T. narismagnus*, 36 years after the last reported record (Hanken 1976: MVZ183028–183035) at the type locality on Volcán San Martín (Hanken and Wake 1998). Additionally, the record from Volcán Santa Marta extends the distribution range of this species 28 km southeast of the closest known locality (Fig. 1). Because the specimens reported here were found in primary vegetation, and Díaz-García et al. (2017) recorded *T. narismagnus* in mature forest but not in restoration areas and cattle pasture in “La Otra Opción” this species is probably not able to survive in disturbed forest or even in moderately disturbed forest. Of the other missing species that the authors have found recently, only one (*T. munificus*) was found in small, highly disturbed forest fragments and within San Juan del Monte state reserve (Juárez-Ramírez et al. 2016), while *Chiropterotriton magnipes* and *C. mosaueri* were found in a cave in a national park with only light to moderate habitat disturbance, and *Pseudoeurycea ahuitzotl* and *P. tlahcuiloh* were found in intact montane forest (AmphibiaWeb 2019).

To more fully understand the conservation status of *T. narismagnus*, an exhaustive sampling effort through time is needed to determine how the encounter rate of this species varies throughout the year and whether it is currently an uncommon species, and to obtain a more accurate estimate of the population size. Extensive fieldwork is also necessary in those areas with favorable environmental conditions on both volcanoes to determine the full distribution of this species. The extent of well-preserved forest on Volcán San Martín is ~100 km², while on Volcán Santa Marta it is ~185 km² (INEGI 2016).

In addition, determining the presence of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) and its possible effect on the survival of these *T. narismagnus* populations is critical, because the presence of *Bd* in Los Tuxtlas region has been confirmed (Mendoza-Almeralla et al. 2015) and this pathogen is suspected of being linked to the population declines of this species (IUCN 2016). The presence of *Bd* has been reported in geographically close species from Central Veracruz, such as *Bolitoglossa rufescens*, *Aquiloerycea cephalica*, and *P. firscheini* (Van Rooij et al. 2011), as well as *P. nigromaculata*

and *Thorius pennatululus*, for which the presence of the pathogen has been associated with declines in their populations (Cheng et al. 2011).

Finally, the finding of two populations of a Critically Endangered salamander species that has gone unrecorded for almost four decades highlights the importance of community and private reserves for harboring species in imminent danger of extinction. Although both reserves are relatively small (less than 200 ha) compared to the Los Tuxtlas Biosphere Reserve in which they are located, most of the land of these reserves is conserved forest surrounded by modified environments. In that sense, this work shows the complementarity between the governmental and non-governmental reserves for the protection of species at risk of extinction (see García-Bañuelos et al. 2019), particularly those species with restricted distributions and sensitivity to environmental disturbances.

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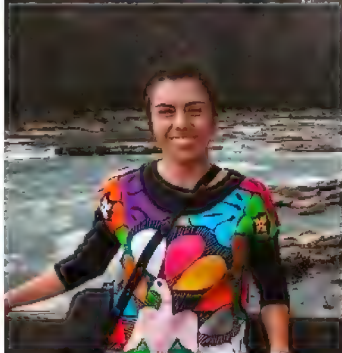
Literature Cited

- AmphibiaWeb. 2019. AmphibiaWeb: Information on Amphibian Biology and Conservation. Berkeley, California, USA. Available: <http://amphibiaweb.org> [Accessed: 20 May 2019].
- Cheng TL, Rovito SM, Wake DB, Vredenburg VT. 2011. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences of the United States of America* 108(23): 9,502–9,507.
- Crump ML, Scott Jr. NJ. 1994. Visual encounters surveys. Pp. 84–92 In: *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Editors, Heyer RW, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS. Smithsonian Institution Press, Washington, DC, USA. 384 p.
- Díaz-García JM, Pineda E, López-Barrera F, Moreno CE. 2017. Amphibian species and functional diversity as indicators of restoration success in tropical montane forest. *Biodiversity and Conservation* 26(11): 2,569–2,589.
- Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5(113): 1–19.
- Frias-Alvarez P, Zúñiga-Vega JJ, Flores-Villela O. 2010. A general assessment of the conservation status and decline trends of Mexican amphibians. *Biodiversity*

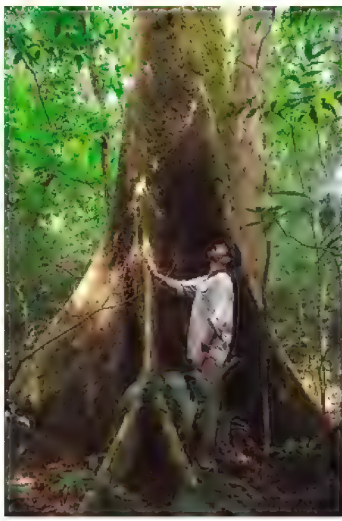
- and Conservation* 19(13): 3,699–3,742.
- García-Bañuelos P, Rovito SM, Pineda E. 2019. Representation of threatened biodiversity in protected areas and identification of complementary areas for their conservation: plethodontid salamanders in Mexico. *Tropical Conservation Science* 12: 1–15.
- Hanken J, Wake DB. 1998. Biology of tiny animals: systematics of the minute salamanders (*Thorius*: Plethodontidae) from Veracruz and Puebla, Mexico, with description of five new species. *Copeia* 1998(2): 312–345.
- Hanken J, Wake DB, Freeman H. 1999. Three new species of minute salamanders (*Thorius*: Plethodontidae) from Guerrero, Mexico, including the report of a novel dental polymorphism in Urodeles. *Copeia* 1999: 917–931.
- INEGI (Instituto Nacional de Estadística y Geografía). 2016. *Conjunto de datos vectoriales de Uso de Suelo y Vegetación escala 1:250 000, Serie VI (capa unión) (ed. 1)*. INEGI, Aguascalientes, Mexico.
- IUCN (International Union for Conservation of Nature). 2019. The IUCN Red List of Threatened Species. Version 2018-2. Available: <http://www.iucnredlist.org>. [Accessed: 11 June 2019].
- IUCN SSC Amphibian Specialist Group. 2016. *Thorius narismagnus*. The IUCN Red List of Threatened Species 2016: e.T59420A53986703. Available: <http://www.iucnredlist.org>. [Accessed: 6 March 2019].
- Juárez-Ramírez MC, Aguilar-López JL, Pineda E. 2016. Protected natural areas and the conservation of amphibians in a highly transformed mountainous region in Mexico. *Herpetological Conservation and Biology* 11(1): 19–28.
- Mendoza-Almeralla C, Burrowes P, Parra-Olea G. 2015. La quitridiomycosis en los anfibios de México. *Revista Mexicana de Biodiversidad* 86(1): 238–248.
- Moritz C, Schneider J, Wake DB. 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. *Systematic Biology* 41(3): 273–291.
- Naturalista. 2019. Salamandra de Cofre de Perote (*Isthmura naucampatepetl*). CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad), Mexico City, Mexico. Available: <https://www.naturalista.mx/taxa/476531-Isthmura-naucFampatepetl>. [Accessed: 6 March 2019].
- Rovito SM, Parra-Olea P, Hanken J, Bonett RM, Wake DB. 2013. Adaptive radiation in miniature: the minute salamanders of the Mexican highlands (Amphibia: Plethodontidae: *Thorius*). *Biological Journal of the Linnean Society* 109(3): 622–643.
- Sandoval-Comte A, Pineda E, Aguilar-López JL. 2012. In search of critically endangered species: the current situation of two tiny salamander species in the Neotropical mountains of Mexico. *PLoS ONE* 7(4): e34023.
- Shannon FA, Werler JE. 1955. Notes on amphibians of the Los Tuxtlas Range of Veracruz, Mexico. *Transactions of the Kansas Academy of Science* 58(3): 360–386.
- Stuart SN, Hoffmann M, Chanson JS, Cox NA, Berridge RJ, Ramani P, Young BE. 2008. *Threatened Amphibians of the World*. Lynx Ediciones, Barcelona, Spain; IUCN, Gland, Switzerland; and Conservation International, Arlington, Virginia, USA. 758 p.
- Swofford DL. 2003. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts, USA.
- Van Rooij P, Martel A, Nerz J, Voitel S, Van Immerseel F, Haesebrouck F, Pasmans F. 2011. Detection of *Batrachochytrium dendrobatidis* in Mexican bolitoglossine salamanders using an optimal sampling protocol. *EcoHealth* 8(2): 237–243.



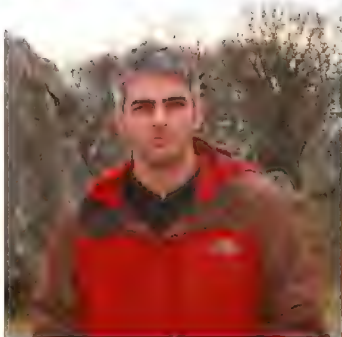
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A new glassfrog (Centrolenidae: *Hyalinobatrachium*) from the Topo River Basin, Amazonian slopes of the Andes of Ecuador

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Abstract.—A new species of glassfrog (Centrolenidae) is described from the San Jacinto River, an affluent of the Topo River, on the Amazonian slopes of the Ecuadorian Andes. The new species, *Hyalinobatrachium adespinosai* sp. nov., can be differentiated from all other centrolenids by the combination of its coloration (transparent peritoneum and pericardium) and vocalization (call duration = 0.38–0.44 s, with 9–13 pulses per call; dominant frequency = 4,645–5,001 Hz). However, *H. adespinosai* sp. nov. is morphologically cryptic with *H. anachoretus*, *H. esmeralda*, and *H. pellucidum*, from which it differs by call traits (in *H. anachoretus*: call duration = 0.32–0.37 s, with 5 or 6 pulses per call, dominant frequency = 4,670–4,800 Hz; in *H. esmeralda*: call duration = 0.218–0.257 s, tonal call, dominant frequency = 4,739–5,580 Hz; in *H. pellucidum*: call duration = 0.112–0.140 s, tonal, dominant frequency = 5,000–5,710 Hz). Biogeographically, the new species is separated from *H. anachoretus* by a considerable distance and, also, the Marañón valley. Finally, following IUCN conservation criteria, the status of the new species is considered as Data Deficient.

Keywords. Amphibia, Anura, Ecuador, Pastaza basin, phylogeny, Tungurahua Province

Resumen.—Describimos una nueva especie de rana de cristal (Centrolenidae) del río San Jacinto, afluente del río Topo, en la vertiente amazónica de los Andes del Ecuador. La especie nueva, *Hyalinobatrachium adespinosai* sp. nov., se diferencia de todos los centrolénidos por la combinación de su coloración ventral (peritoneo y pericardio transparentes) y las características de su canto (duración del canto = 0.382–0.430 s, con 9–13 pulsos por canto; frecuencia dominante = 4,645–5,001 Hz). Sin embargo, es morfológicamente críptica con *H. anachoretus*, *H. esmeralda* y *H. pellucidum*, especies de las cuales difiere por su canto (en *H. anachoretus*: duración del canto = 0.32–0.37 s, con 5 or 6 pulsos por canto, frecuencia dominante = 4,670–4,800 Hz; en *H. esmeralda*: duración del canto = 0.218–0.257 s, tonal, frecuencia dominante = 4,739–5,580 Hz; en *H. pellucidum*: duración del canto = 0.112–0.140 s, tonal, frecuencia dominante = 5,000–5,710 Hz). Finalmente, siguiendo los criterios de la UICN, sugerimos que *Hyalinobatrachium adespinosai* sp. nov. sea ubicada en la categoría de Datos Insuficientes.

Palabras clave. Amphibia, Anura, Cuenca del Pastaza, Ecuador, filogenia, Tungurahua Province

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Introduction

The glassfrog genus *Hyalinobatrachium* (sensu Ruiz-Carranza and Lynch 1991, as modified by Guayasamin et al. 2009) is one of the most charismatic anuran groups because of its peculiar morphological and behavioral

traits. One of the most striking traits of this genus is its complete ventral transparency, produced by having a transparent ventral peritoneum (Ruiz-Carranza and Lynch 1991; Cisneros-Heredia and McDiarmid, 2007; Guayasamin et al. 2009). Males of all species in this genus also exhibit extended parental care towards

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fertilized egg clutches deposited on the leaves of trees (see Delia et al. 2017). Extended male parental care, a derived trait that has evolved at least twice in glassfrogs, is coupled with egg deposition on the underside of leaves in all *Hyalinobatrachium* and some *Centrolene* species (Ruiz-Carranza and Lynch 1991; Guayasamin et al. 2009; Delia et al. 2017; Salgado and Guayasamin 2018).

Although assigning species to *Hyalinobatrachium* is straightforward, distinguishing among members of this genus is more complicated because species of *Hyalinobatrachium* tend to be remarkably similar, both morphologically and ecologically. In recent years, species discovery in frogs has relied heavily on molecular and acoustic traits (Castroviejo-Fisher et al. 2009, 2011; Kubicki et al. 2015; Guayasamin et al. 2017). Calls, in particular, are especially useful for distinguishing among cryptic species since they function as efficient prezygotic mating recognition signals (Narins and Capranica 1976; Duellman and Trueb 1994; Zakon and Wilczynski 1988; Wilczynski and Ryan 1999; Wells 2007). As previously demonstrated in glassfrogs, the acoustic differences between species are often more pronounced than morphological differences (Escalona-Sulbarán et al. 2018).

We describe a new species of *Hyalinobatrachium* that is morphologically cryptic with *H. anachoretus* Twomey, Delia, and Castroviejo-Fisher 2014, *H. esmeralda* Ruiz-Carranza and Lynch 1998, and *H. pellucidum* (Lynch and Duellman 1973). The new species, which is known from a single locality in the Topo River basin, is differentiated from these two species by its call and genetics.

Materials and Methods

Ethics statement. Research was conducted under permits NoMAE-DNB-CM-2015-2017, 019-2018-IC-FAU-DNB/MAE, and 018-2017-IC-FAU-DNB/MAE, issued by the Ministerio del Ambiente del Ecuador. The study was carried out in accordance with the guidelines for use of live amphibians and reptiles in field research (Beaupre et al. 2004), compiled by the American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists' League (HL), and the Society for the Study of Amphibians and Reptiles (SSAR).

Taxonomy and species concept. Glassfrog generic and family names follow the taxonomy proposed by Guayasamin et al. (2009). Species are considered as segments of separately evolving metapopulation lineages, following the conceptual framework developed by Simpson (1951, 1961), Wiley (1978), and de Queiroz (2007). Assessing whether a given population is an independent lineage is a non-trivial task, especially when working with closely related taxa. In such cases, analyzing many different sets of characters provides tools for supporting species hypotheses (Dayrat 2005; de Queiroz 2007; Padial et al. 2009).

Morphological data. Lynch and Duellman (1973) and Cisneros-Heredia and McDiarmid (2007) are followed for the diagnosis and description of the new species. The webbing formula follows Savage and Heyer (1967), as

modified by Guayasamin et al. (2006). The taxonomy of centrolenid frogs follows the proposal by Guayasamin et al. (2009). Comparisons were made between various *Hyalinobatrachium* specimens (see Appendix 1) housed at the following collections: Instituto de Ciencia Naturales, Universidad Nacional de Colombia, Bogotá, Colombia (ICN); University of Kansas, Museum of Natural History, Division of Herpetology, Lawrence, Kansas, USA (KU); Museo de Zoología, Universidad Tecnológica Indoamérica, Quito, Ecuador (MZUTI); National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM); and Museo de Zoología, Universidad San Francisco de Quito, Quito, Ecuador (ZSFQ). Morphological measurements were taken with a Mitutoyo® digital caliper to the nearest 0.1 mm, as described by Guayasamin and Bonaccorso (2004), except when noted, and are as follows: (1) snout–vent length (SVL); (2) tibia length; (3) foot length; (4) head length; (5) head width; (6) interorbital distance (IOD); (7) upper eyelid width; (8) internarial distance; (9) eye-to-snout distance; (10) eye diameter; (11) tympanum diameter; (12) radioulna length; (13) hand length; (14) Finger I length; (15) Finger II length = distance from outer margin of palmar tubercle to tip of Finger II; and (16) width of disc of Finger III. Sexual maturity was determined by the presence of vocal slits and calling activity in males.

Bioacoustics. Sound recordings were made with an Olympus LS-10 Linear PCM Field Recorder and a Sennheiser K6–ME 66 unidirectional microphone. The calls were recorded in WAV format with a sampling rate of 44.1 kHz/s with 16 bits/sample. Measurements and definitions of acoustic variables follow Köhler et al. (2017). Notes were divided into two classes—“pulsed” and “tonal”—based upon the distinct waveforms on the oscillogram (see Hutter and Guayasamin 2012). Pulsed (also termed peaked) notes are defined as those with one or more clear amplitude peaks and amplitude modulation (i.e., visible increases and decreases in amplitude on the oscillogram throughout the call). In contrast, tonal notes are defined as those with no clear amplitude peak (Dautel et al. 2011). In this study the call of *Hyalinobatrachium pellucidum* (Lynch and Duellman 1973) is also described from an individual (USNM 286708) recorded at the type locality of the species (Río Azuela, 0.1167°S, 77.617°W, 1,740 m, Napo province, Ecuador) by Roy McDiarmid; the recording is deposited at the Cornell University Macaulay Library (ML Catalogue No. 202401).

Evolutionary relationships. Mitochondrial sequences (16S) were generated for four individuals (ZSFQ 1647–48, 1650–51) of the new species of *Hyalinobatrachium*. Extraction, amplification, and sequencing protocols were as described in Guayasamin et al. (2008). The sequences obtained were compared with all those available for other species of glassfrogs (family Centrolenidae) and its sister taxon Allophrynidae (see Austin et al. 2002; Guayasamin et al. 2018). These sequences were downloaded from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), and were generated mostly by Guayasamin et al. (2008), Castroviejo-Fisher et al. (2014), and Twomey et al.

(2014), but also included data from the newly described *H. yaku* Guayasamin et al. 2017 and *H. muiraquitana* de Oliveira and Hernández-Ruz 2017. Sequences were aligned using MAFFT v.7 (Multiple Alignment Program for Amino Acid or Nucleotide Sequences: <http://mafft.cbrc.jp/alignment/software/>), with the Q-INS-i strategy. MacClade 4.07 (Maddison and Maddison 2005) was used to visualize the alignment (no modifications were necessary). Maximum likelihood (ML) was run in the IQ-TREE 1.5.5 software (Nguyen et al. 2015). The best-fitting nucleotide substitution model was implemented using ModelFinder within IQ-TREE (Kalyaanamoorthy et al. 2017), which groups partitions with the same model and similar rates, and simultaneously searches the model and tree space. Node support was assessed via 1,000 ultra-fast bootstrap replicates, a method that shows less bias than other support estimates (Minh et al. 2013). Ultra-fast bootstrapping also leads to straightforward interpretation of the support values (e.g., support of ≥ 95 bootstrap should be interpreted as significant; Minh et al. 2013).

Results

Phylogenetic relationships. Based on the Bayesian Information Criterion, the best-fit model for this dataset was GTR+F+R5. Rate parameters were estimated as follows: A–C: 2.690, A–G: 9.067, A–T: 3.078, C–G: 0.346, C–T: 23.835, and G–T: 1.000. Base frequencies were: A = 0.346, C = 0.239, G = 0.181, and T = 0.234.

The phylogeny (Fig. 1) confirms the placement of the new species within the genus *Hyalinobatrachium*. The new species, described below, is inferred as part of a clade formed by four species that have very similar morphologies: the new species (described below) + *H. anachoretus* + *H. pellucidum* + *H. yaku*.

Species description

Hyalinobatrachium adespinosai new species

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Suggested English name: Adela's Glassfrog

Suggested Spanish name: Rana de Cristal de Adela

Holotype. ZSFQ 1648 (JMG 583, Fig. 2), adult male from riverine vegetation along the San Jacinto River (1.3447°S, 78.1814°W; 1,795 m asl), Tungurahua Province, Ecuador, collected by CRH, REG, and KC on 4 August 2017.

Paratypes. ZSFQ 1650–52, 1647, adult males with same data as holotype.

Generic placement. The new species is placed in the genus *Hyalinobatrachium* (Ruiz-Carranza and Lynch, 1991, as modified by Guayasamin et al. 2009) on the basis of morphological and molecular data. The main diagnostic phenotypic traits of *Hyalinobatrachium* are: (1) ventral parietal peritoneum completely transparent; (2) digestive tract and bulbous liver covered by iridophores; (3) humeral spines absent; (4) white bones

in life; (5) males call from the underside of leaves; (6) females place the eggs on the underside of leaves; and (7) males provide extended parental care. All the aforementioned characteristics are shared by the new species. Additionally, analyses of the mitochondrial 16S gene place the new species as a close relative of other *Hyalinobatrachium* species (Fig. 1); thus, generic placement in *Hyalinobatrachium* is unambiguous.

Diagnosis. Within the genus *Hyalinobatrachium*, the new species is diagnosable mainly by having a transparent pericardium. However, the new species is morphologically cryptic with three closely related taxa (*H. anachoretus*, *H. pellucidum*, *H. esmeralda*). Based on comparisons with specimens examined (see Appendix 1), all these species display a similar size and color pattern (pale green dorsum with yellow dots and a transparent venter and pericardium; red heart visible ventrally). However, calls between species diverge noticeably; the major difference is the structure of the call, with two species (*H. adespinosai* **sp. nov.** and *H. anachoretus*) having pulsed calls and the others having tonal vocalizations (Fig. 3; Table 1). The call of *H. adespinosai* **sp. nov.** is further differentiated from that of *H. anachoretus* by being longer, having more pulses per note, and being produced at a higher rate (Table 1). Toe webbing (Toe IV) is less extensive in the new species ($2^{1/3}$ IV 2^+) than in *H. anachoretus* (1^+ IV 1^+ ; Twomey et al. 2014). Additionally, the new species and *H. anachoretus* are separated by considerable distance (airline distance = 473 km), including one of the most important biogeographic barriers in South America, the Marañón valley (see Duellman 1999; Winger and Bataillon 2015 and references therein). Uncorrected *p* genetic distances for the mitochondrial gene 16S between *H. adespinosai* **sp. nov.** and its closest relatives are summarized in Table 2.

Characterization. The following combination of characters is found in *Hyalinobatrachium adespinosai* **sp. nov.**: (1) dentigerous process of the vomer lacking teeth; (2) snout truncate in dorsal and lateral views; (3) tympanum barely visible, hidden under skin, with coloration similar to that of surrounding skin; (4) dorsal skin shagreen; (5) ventral skin areolate; cloacal ornamentation absent, paired round tubercles below vent absent; (6) parietal peritoneum transparent; pericardium with thin layer of iridophores (in life, a red heart is mostly visible ventrally); liver, viscera, and testes covered by iridophores; (7) liver bulbous; (8) humeral spines absent; (9) hand webbing formula: I (2–3) — (2–2⁺) II (1–1⁺) — $3^{1/3}$ III (2–2⁺) — (2–2) IV; (10) foot webbing moderate; webbing formula: I 1 — ($1^{2/3}$ –2[–]) II (1–1[–]) — (2–2^{1/3}) III (1–1⁺) — (2⁺–2^{1/3}) IV 2⁺ — (1⁺–1^{1/3}) V; (11) fingers and toes with thin lateral fringes; ulnar and tarsal folds present, but low and difficult to distinguish, with thin layer of iridophores that extends to ventrolateral edges of Finger IV and Toe V; (12) nuptial excrescence present as a small pad on Finger I (Type V), prepollex not enlarged; prepollical spine not projecting (spine not exposed); (13) when appressed, Finger I longer than II; (14) diameter of eye about two times wider than disc on

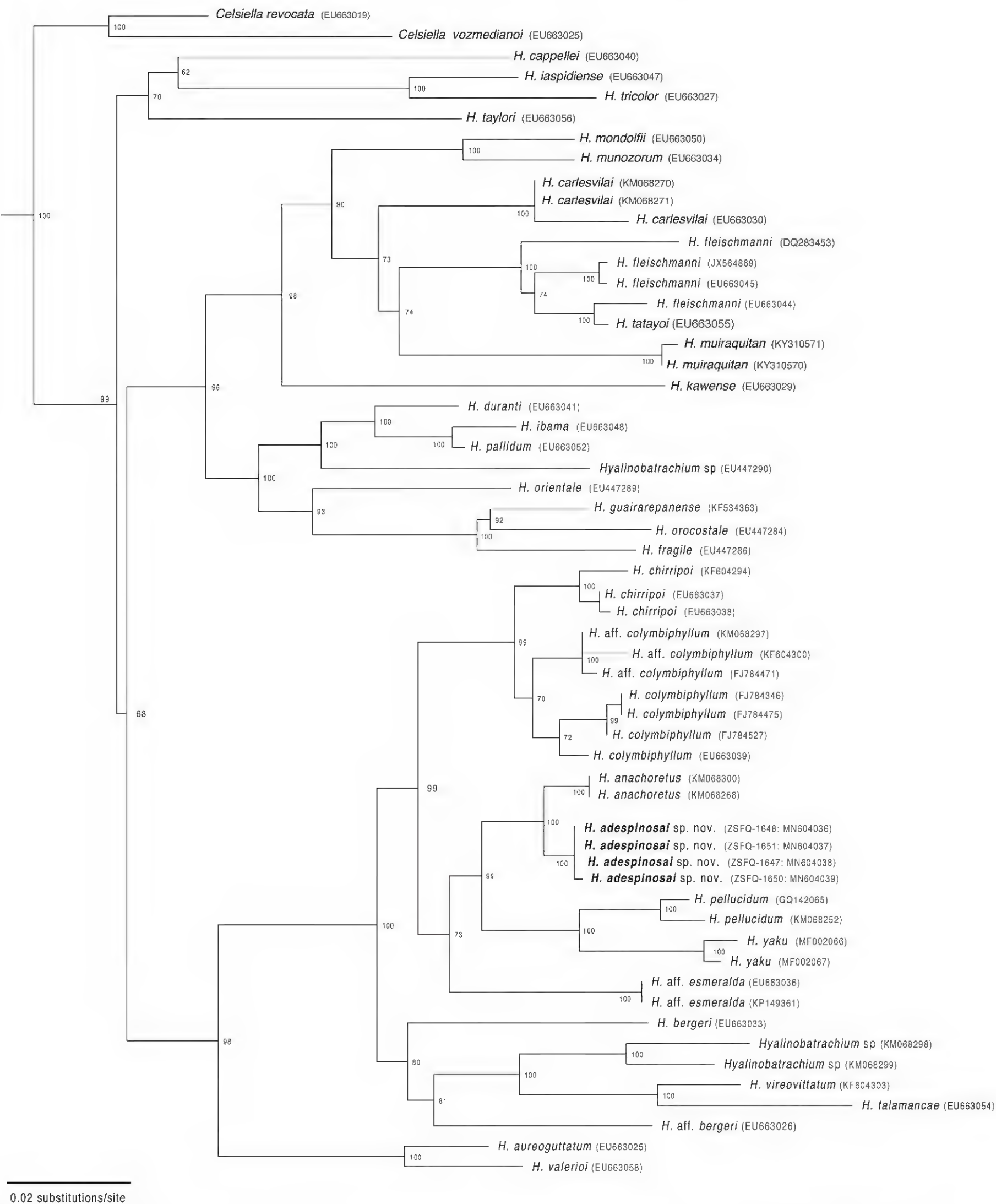


Fig. 1. Phylogenetic relationships of *Hyalinobatrachium* inferred from the 16S mitochondrial gene under ML criteria. All sequences were downloaded from GenBank, except for those of the new species. GenBank codes are listed next to each terminal. Associated locality data is available at GenBank, as well as in Guayasamin et al. (2008), Castroviejo-Fisher et al. (2014), and Twomey et al. (2014).

Finger III; (15) coloration in life: dorsal surfaces pale yellowish green with small pale yellow spots and minute gray to black melanophores; bones white; (16) coloration in preservative: dorsal surfaces pale cream with minute melanophores; (17) iris coloration in life: white with pale yellow hue and numerous minute lavender spots; (18) melanophores absent from most fingers and toes, but present on Finger IV and Toes IV and V; (19) males call

from underside of leaves; advertisement call consisting of single note, distinctly pulsed (9–13 pulses per call), with duration of 0.382–0.430 s, and dominant frequency at 4,645–5,001 Hz; (20) males attend egg clutches located on the underside of leaves overhanging streams; clutch size of 22 embryos ($n = 1$); (21) SVL in adult males 20.5–22.2 mm ($n = 3$), females unknown; and (22) enameled tubercles absent from sides of head.



Fig. 2. *Hyalinobatrachium adespinosai* sp. nov. in life, holotype.

Description of the holotype. ZSFQ 1648, adult male with SVL 22.2 mm. Head wider than long (head width 38% of SVL; head length 77% of head width). Snout truncate in dorsal and lateral views. Loreal region flat and nearly vertical, nostrils slightly protuberant, elliptical; internarial region concave anterodorsally; canthus rostralis well defined. Eyes small, directed anterolaterally, eyes about 45° relative to midline (where

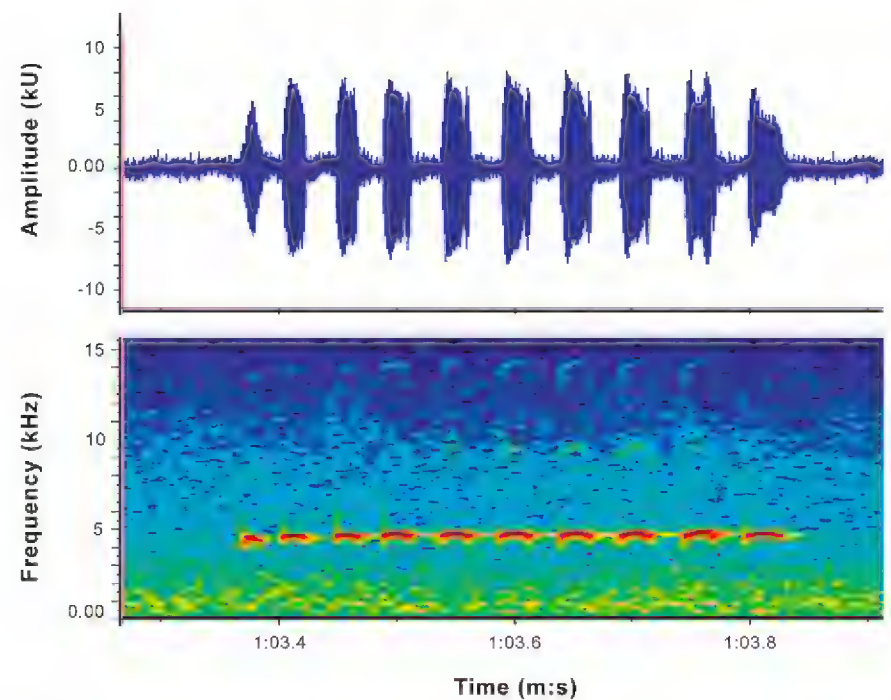


Fig. 3. Call of *Hyalinobatrachium adespinosai* sp. nov., holotype, recorded in field conditions at the type locality. Air temperature: 18 °C.

anterior-facing eyes would be 90° relative to midline). Tympanum annulus barely visible through the skin; tympanic membrane differentiated and pigmented as surrounding skin. Dentigerous processes on vomers absent; choanae large, oval, separated widely (distance about the same as between nostrils); tongue round, white in preservative, anterior 4/5 attached to mouth; vocal slits present, extending along floor of mouth lateral to tongue; enameled glands absent from lower part of upper jaw. Ulnar fold present, with a thin layer of iridophores, and continuing along the external edge of Finger IV; humeral spine absent. Relative lengths of fingers: $I < II < IV < III$; finger discs rounded, about the same size as discs on toes, disc on Finger III 54% of eye width; finger webbing reduced between Fingers I–III, moderate between Fingers III and IV, with formula $I\ 3-2^+ II\ 1^+ - 3^{1/3} III\ 2 - 2 IV$. Prepollex concealed; subarticular tubercles round, faint; few small supernumerary tubercles present, palmar tubercle round and small, thenar tubercle ovoid; nuptial excrescences present as a small pad on external edge of Finger I (Type V). Hind limbs slender, tibia length 58% of SVL; tarsal fold present, with thin layer of iridophores; discs of toes round, inner metatarsal tubercle small; outer metatarsal tubercle round, but very difficult to distinguish. Webbing formula of feet: $I\ 1 - 2^{1/3} II\ 1 - 2^{1/3} III\ 1^+ - 2^{1/3} IV - 2^+ - 1V$. In preservative, dorsal skin peppered with small dark lavender melanophores; dorsal skin shagreen; skin on venter areolate; cloacal opening at level of upper thighs, cloacal ornamentation present as a lightly enameled cloacal fold and small tubercles. Parietal peritoneum transparent; pericardium with a very thin layer of iridophores that, in life, exposes a red heart; urinary bladder lacking iridophores; liver, viscera, and testes fully covered by iridophores. Kidneys rounded, approximately bean-shaped; liver bulbous.

Coloration in life. Dorsal surfaces apple green to yellowish green with diffuse yellow spots and minute gray to black melanophores. Melanophores absent from fingers and toes, except Finger IV and Toes IV and V. Ventrally, parietal peritoneum and pericardium transparent, with a red heart always visible, even

Table 1. Comparison among calls of closely related species of *Hyalinobatrachium*. The calls of all species were recorded at the corresponding type locality, except for that of *H. esmeralda*. Call data were obtained from the following sources: *H. anachoretus* from Twomey et al. (2014), *H. esmeralda* from Acosta-Galvis (2017), and *H. yaku* from Guayasamin et al. (2017).

Species	Distribution	Sample size: # of individuals / # of calls	Call duration (s) range (mean and standard deviation)	# notes per call	Call structure	# pulses per call	Time between calls (s)	Frequency modulation	Dominant frequency at beginning of call (hz)	Dominant frequency at end of call (hz)	1st harmonic (hz)
<i>H. adespinosai</i> sp. nov.	Ecuador: Amazonian slopes of the Andes, 1,670–1,795 m	9/70	0.38–0.44 (x̄ = 0.38 ± 0.017)	1	Pulsed	9–13 (x̄ = 10.49 ± 1.281)	2.0–11.0 (x̄ = 4.58 ± 2.317)	No	4,645–5,203 (x̄ = 4,855 ± 152)	Same as at beginning	9,336–9,380
<i>H. anachoretus</i>	Peru: Amazonian slopes of the Andes, 2,001–2,050 m	1/4	0.32–0.37	1	Pulsed	5–6	13–16	No	4,670–4,800	Same as at beginning	No
<i>H. esmeralda</i>	Colombia: Amazonian slopes of the Andes, 1,026–1,700 m	1/14	0.218–0.257 (x̄ = 0.239 ± 0.014)	1	Tonal	1 (x̄ = 1 ± 0)	4.7–31.1 (x̄ = 11.82 ± 7.01)	Yes, very subtle increase	4,739–5,581 (5,204 ± 298.2)	Slightly higher	No
<i>H. pellucidum</i>	Ecuador, Peru: Amazonian slopes of the Andes, 523–1,740 m	1/13	0.11–0.14 (x̄ = 0.13 ± 0.009)	1	Tonal	1–3 (x̄ = 2.15 ± 0.009)	2.3–3.3 (x̄ = 2.7 ± 0.3)	Yes, very subtle increase	5,000–5,719 (x̄ = 5,263 ± 207.1)	Slightly higher	11,213–11,721
<i>H. yaku</i>	Ecuador: Amazonian lowlands, 300–360 m	1/10	0.27–0.40 (x̄ = 0.3 ± 0.03)	1	Tonal	1 (x̄ = 1 ± 0)	5.3–8.9 (x̄ = 7.1 ± 1.1)	No	5,219–5,330 (x̄ = 5,284 ± 35.0)	Same as at beginning	No

when a very thin layer of iridophores is present on the pericardium of some individuals. Visceral peritoneum of gall bladder and urinary bladder transparent; hepatic and visceral peritonea white; ventral vein red. Iris pale yellowish white, with numerous minute lavender spots. Bones white.

Coloration in preservative. Dorsal surfaces cream, dotted with minute dark lavender melanophores; venter uniform cream; visceral peritoneum lacking iridophores; pericardium with a very thin layer of iridophores. Iris silvery white with minute lavender melanophores.

Measurements. Measurements of the type series are shown in Table 3.

Variation. Variation in hand webbing is as follows: I (2–3) — (2–2⁺) II (1–1⁺) — 3^{1/3} III (2–2⁺) — (2–2) IV. Foot webbing variation is as follows: I 1— (1^{2/3}–2[–]) II (1–1[–]) — (2–2^{1/3}) III (1–1⁺) — (2⁺–2^{1/3}) IV 2⁺ — (1⁺–1^{1/3}) V.

Vocalizations (Fig. 3). The description is based on recordings from nine individuals (Codes LBE-C: 048–057). The call of *Hyalinobatrachium adespinosai* sp. nov. has a striking resemblance to the chirp of a cricket, and was often confused for one in the field. Each call is composed of a single and high-pitched pulsed note, and has a duration of 0.38–0.44 s (x̄ = 0.38 ± 0.017). Time between calls varied from 2.0–11.0 s (x̄ = 4.58 ± 2.3). The fundamental frequency, the same as the dominant frequency, is at 4,645–5,203 Hz (x̄ = 4,855 ± 152). There is no frequency modulation. The first harmonic is at 9,336–9,754 Hz and the second harmonic is at 14,159–14,444 Hz.

Ecology. All individuals of the new species were found on the underside of leaves of riverine vegetation along the San Jacinto River. The section of river was fast-flowing and had visible rapids. Although the population is locally abundant (as heard from numerous advertisement calls), individuals are very difficult to observe because they are usually found at the canopy level (4–16 m above ground level). The type series consists of males exclusively; they were calling in the months of July and August. One male (ZSFQ 1648) was apparently guarding an egg clutch containing 22 embryos; both the adult male and the egg clutch were on the same leaf most of the time, but the male also moved to nearby leaves (Fig. 4).

Distribution. *Hyalinobatrachium adespinosai* sp. nov. is only known from the type locality: San Jacinto River (1.3447°S, 78.1814°W; 1,795 m asl), Tungurahua Province, Ecuador (Fig. 5).

Evolutionary relationships. The phylogenetic analyses recover *Hyalinobatrachium adespinosai* sp. nov. haplotypes as sister to haplotypes sampled from *H. anachoretus* and nested within other members of a monophyletic clade comprised of all other sampled species of *Hyalinobatrachium* (Fig. 1). The most closely related species to *H. adespinosai* sp. nov. share several

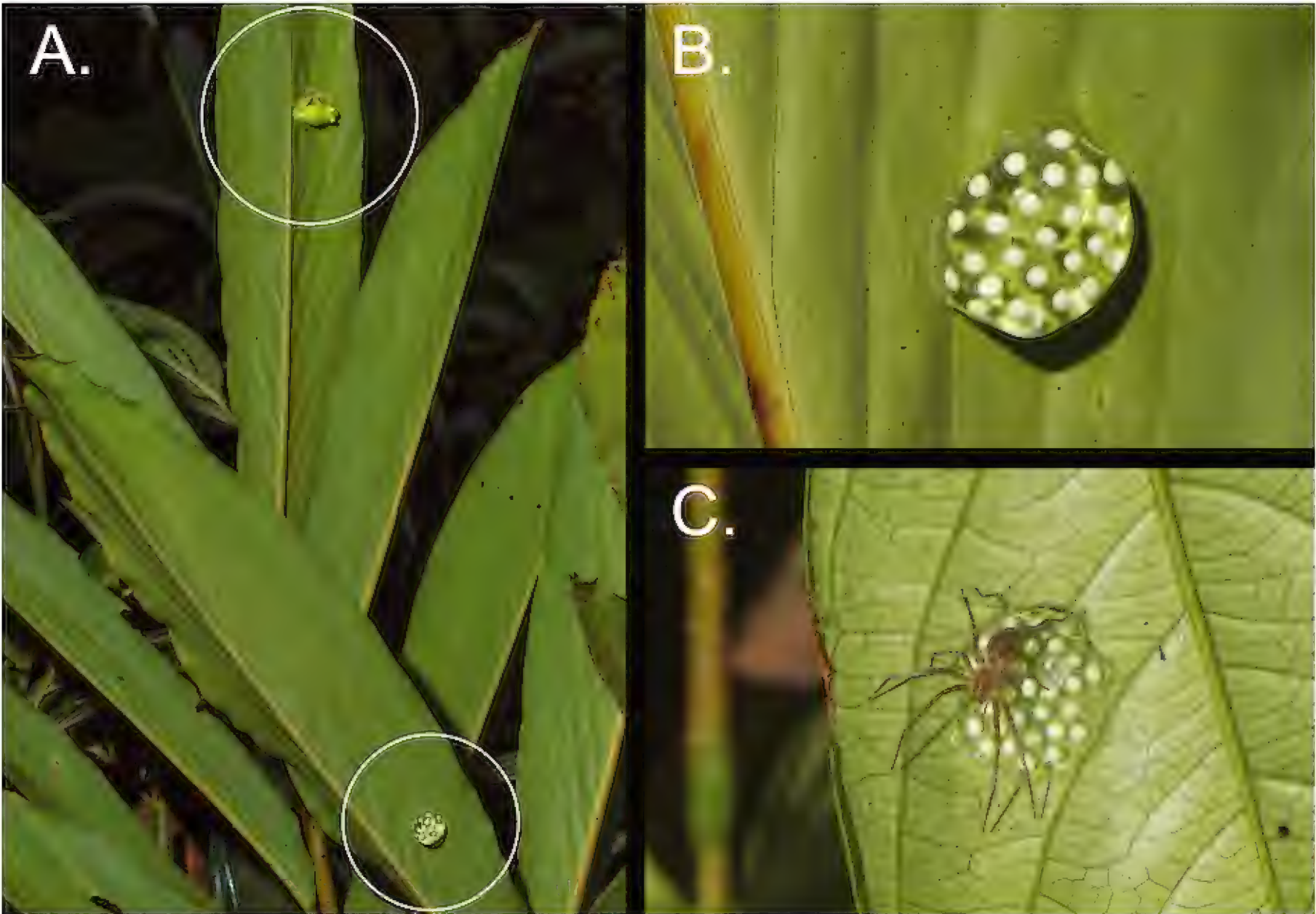


Fig. 4. Natural history and in-situ photographs of the new species. (A) Adult male of *Hyalinobatrachium adespinosai* near his egg clutch; other males were observed on the same leaf as the egg clutch. (B) Close-up of the egg clutch. (C) Spider predation on an unattended egg clutch.

morphological traits, including a red heart exposed ventrally (*H. adespinosai* + *H. anachoretus* + *H. pellucidum* + *H. yaku*).

Etymology. The specific epithet *adespinosai* honors Adela Espinosa, an Ecuadorian conservationist and board member of the Jocotoco Foundation (<http://www.jocotoco.org>). Adela’s work has focused on the conservation of species and ecosystems. The new glassfrog described here is found only within the limits of a natural reserve owned by Adela and her husband, Antonio Páez. We are delighted to recognize Adela’s devotion to nature with this marvelous species.

Conservation status. Available information is insufficient to fully assess the conservation status of *Hyalinobatrachium adespinosai* **sp. nov.** Therefore, following IUCN criteria, this species is considered as Data Deficient. The herpetological museums that house

specimens collected near the type locality (Topo basin) were consulted, but there were no additional specimens of the new species. Although this might suggest a conservation category other than Data Deficient, we actually prefer to maintain this status because the new species is very difficult to find (i.e., a canopy specialist). Therefore, in this case, absence in nearby localities where herpetological surveys have been carried out does not necessarily indicate a true absence of the species.

Discussion

Morphological stasis is expected in species under similar ecological conditions, whereas traits associated with social signaling tend to evolve more rapidly (Winger and Bator 2015; Arnegard et al. 2010; Safran et al. 2013; Escalona-Sulbarán et al. 2018). Species in the glassfrog genus *Hyalinobatrachium* exhibit a striking morphological homogeneity (see Ruiz-Carranza and

Table 2. Genetic distances (uncorrected *p* matrix for 16S, 813 base pairs) between *Hyalinobatrachium adespinosai* **sp. nov.** and closely related species.

	<i>H. adespinosai</i>	<i>H. anachoretus</i>	<i>H. esmeralda</i>	<i>H. pellucidum</i>	<i>H. yaku</i>
<i>H. adespinosai</i>	0.000–0.001				
<i>H. anachoretus</i>	0.010–0.011	0.000			
<i>H. esmeralda</i>	0.0254–0.0272	0.0272	0.000		
<i>H. pellucidum</i>	0.032–0.034	0.029–0.037	0.034–0.040	0.007–0.009	
<i>H. yaku</i>	0.036–0.037	0.033–0.041	0.038–0.040	0.025–0.030	0.000–0.001



Fig. 5. Distribution of *Hyalinobatrachium adespinosai* sp. nov. in Ecuador.

Lynch 1998; Guayasamin et al. 2009; Castroviejo-Fisher et al. 2011), perhaps because of the constraints associated not only with their similar ecology, but also with their derived reproductive strategy (prolonged parental care on the underside of leaves). The obvious consequence is that traditional morphological trait-based criteria provide an underestimation of the true biological diversity of the genus. In contrast, call traits in centrolenids have shown more variation than morphology (Escalona-Sulbarán et al. 2018). Acoustic signals can diverge because of the effects of multiple mechanisms, including drift (i.e., isolation-by-distance), natural selection (i.e., adaptation to local ecological conditions, reinforcement, character displacement), and/or sexual selection (i.e., sensory exploitation, divergent female choice; reviewed by Wilczynski and Ryan 1999; Wells 2007; Prum 2017; Köhler et al. 2017). However, this study represents another example of how vocalizations can be extremely useful for species discovery.

Given the lack of information for *Hyalinobatrachium adespinosai* sp. nov., we consider the species as Data Deficient, following the IUCN criteria. The species is

locally abundant at the type locality and as currently known has a restricted distribution. However, given that the species is usually found at the canopy level, it is extremely difficult to locate individuals of this species, so we cannot infer its true distribution based solely on the lack of prior collection.

Establishing clear biogeographic patterns in groups where new species are often being described is challenging. However, *Hyalinobatrachium* species are generally found in the lowlands while *Centrolene* and *Nymphargus* species are predominantly Andean (Guayasamin et al. 2009; Hutter et al. 2017). In contrast to these general patterns, the clade formed by *H. adespinosai* sp. nov. + *H. anachoretus* + *H. pellucidum* + *H. yaku* + *H. esmeralda* is mostly found on the Amazonian slopes of the Andes (except *H. yaku*). Since tropical species tend to have narrow thermal niches (Shah et al. 2017, Polato et al. 2018), the linearity of the Andean mountain range might promote speciation by reducing contact and gene flow among parapatric populations (see Fig. 6), as suggested by Graves (1988). Similar patterns (i.e., closely related species along the same slope of

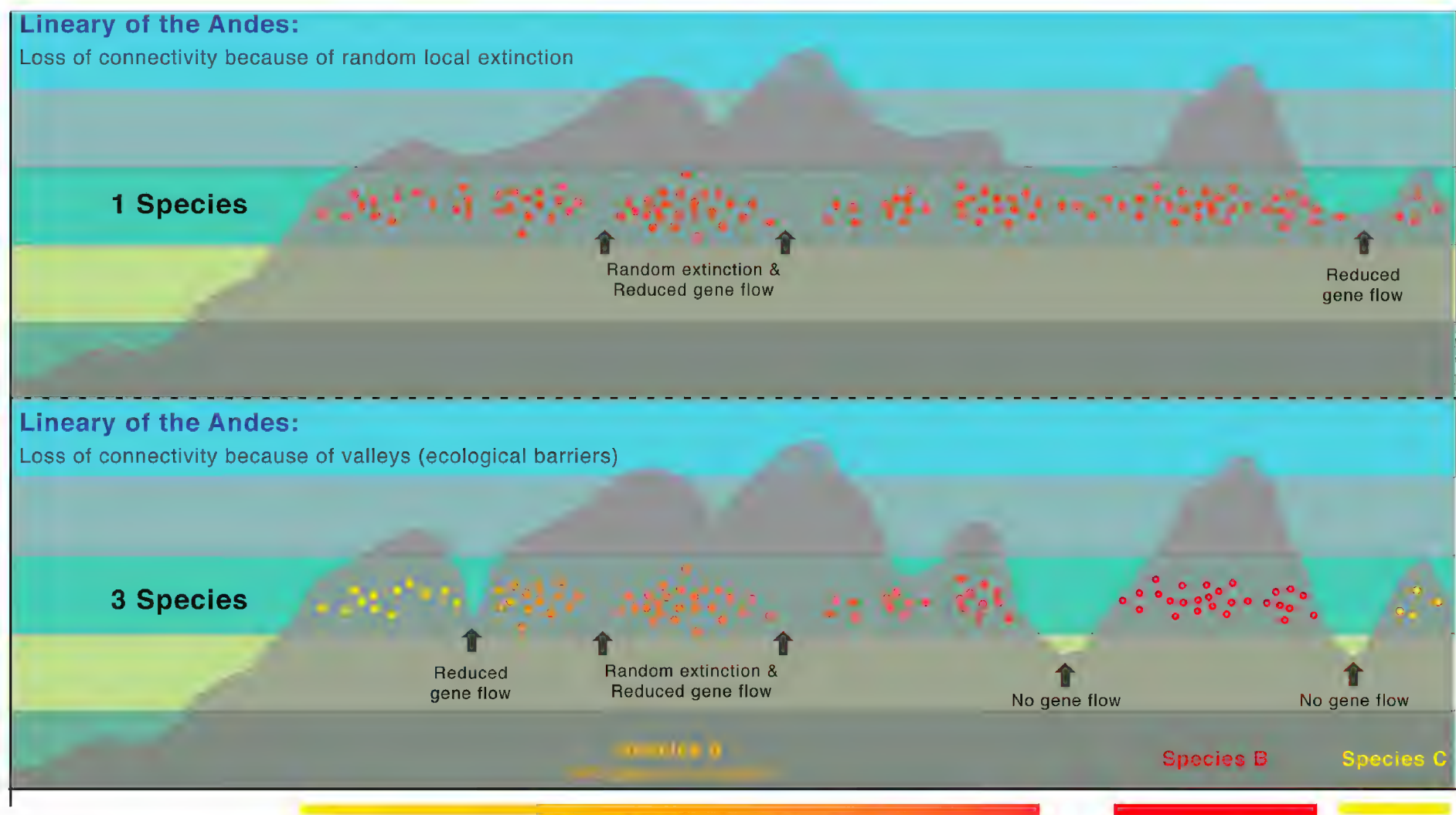


Fig. 6. Schematic graph illustrating how the linearity of the Andes facilitates the speciation process.

the Andes) have also been observed in other glassfrogs (e.g., *Nymphargus*; Guayasamin et al. 2019) and birds (Bonaccorso 2009; Benham et al. 2015; Cadena et al. 2019).

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Literature Cited

Arnegard ME, McIntyre PB, Harmon LJ, Zelditch ML, Crampton WGR, Davis JK, Sullivan JP, Lavoue S, Hopkins CD. 2010. Sexual signal evolution outpaces ecological divergence during electric fish species radiation. *American Naturalist* 176: 335–356.

Austin JD, Loughheed SC, Tanner K, Chek AA, Bogart JP, Boag PT. 2002. A molecular perspective on the evolutionary affinities of an enigmatic Neotropical frog, *Al-*

Table 3. Meristic variation of *Hyalinobatrachium adespinosai* sp. nov. (in mm).

	ZSFQ 1647 (holotype)	ZSFQ 1651	ZSFQ 1647
Sex	Male	Male	Male
SVL	22.2	20.7	20.5
Femur	12.8	12.0	12.1
Tibia	12.8	11.5	11.9
Foot	11.0	10.5	10.5
Head length	6.5	6.4	6.2
Head width	8.4	7.7	7.9
IOD	2.8	2.5	2.6
Upper eyelid	1.6	1.4	1.6
Internarinal distance	1.8	1.7	1.7
Eye diameter	2.6	2.3	2.3
Eye-to-snout distance	3.2	2.8	3.0
Tympanum diameter	0.7	0.7	0.7
Radioulna	5.5	4.8	5.2
Hand length	6.7	6.4	6.4
Finger I	4.9	4.7	4.8
Finger II	4.5	4.4	4.5
Disc Finger III	1.4	1.2	1.2
Disc Toe IV	1.3	1.2	1.2

- lophryne ruthveni*. *Zoological Journal of the Linnean Society* 134: 335–346.
- Benham PM, Cuervo AM, McGuire JA, Witt CC. 2015. Biogeography of the Andean metaltail hummingbirds: contrasting evolutionary histories of tree line and habitat-generalist clades. *Journal of Biogeography* 42: 763–777.
- Benham PM, Witt CC. 2016. The dual role of Andean topography in primary divergence: functional and neutral variation among populations of the hummingbird, *Metallura tyrianthina*. *BMC Evolutionary Biology* 16: 22.
- Bonaccorso E. 2009. Historical biogeography and speciation in the Neotropical highlands: molecular phylogenetics of the jay genus *Cyanolyca*. *Molecular Phylogenetics and Evolution* 50: 618–632.
- Cadena CD, Pérez-Emán JL, Cuervo AM, Céspedes LN, Epperly KL, Klicka JT. 2019. Extreme genetic structure and dynamic range evolution in a montane passerine bird: implications for tropical diversification. *Biological Journal of the Linnean Society* 126: 487–506.
- Castroviejo-Fisher S, Padial JM, Chaparro JC, Aguayo R, de la Riva I. 2009. A new species of *Hyalinobatrachium* (Anura: Centrolenidae) from the Amazonian slopes of the central Andes with comments on the diversity of the genus in the area. *Zootaxa* 2143: 24–44.
- Castroviejo-Fisher S, Vilà C, Ayarzagüena J, Blanc M, Ernst R. 2011. Species diversity of *Hyalinobatrachium* glassfrogs (Amphibia: Centrolenidae) from the Guiana Shield, with the description of two new species. *Zootaxa* 3132: 1–55.
- Castroviejo-Fisher S, Guayasamin JM, Gonzalez-Voyer A, Vilá C. 2014. Neotropical diversification seen through glassfrogs. *Journal of Biogeography* 41: 66–80.
- Cisneros-Heredia DF, McDiarmid RW. 2007. Revision of the characters of Centrolenidae (Amphibia: Anura: Athesphatanura), with comments on its taxonomy and the description of new taxa of glassfrogs. *Zootaxa* 1572: 1–82.
- Dautel N, Salgado Maldonado AL, Abuza R, Imba H, Griffin K, Guayasamin JM. 2011. Advertisement and combat calls of the glass frog *Centrolene lynchi* (Anura: Centrolenidae), with notes on combat and reproductive behavior. *Phyllomedusa* 10: 31–43.
- Dayrat B. 2005. Toward integrative taxonomy. *Biological Journal of the Linnean Society* 85: 407–415.
- de Oliveira EA, Hernández-Ruz EJ. 2017. New species of glassfrog, genus *Hyalinobatrachium* (Anura: Centrolenidae), for the Brazilian Amazon revealed by mitochondrial DNA and morphology. *International Journal of Research Studies in Biosciences* 5: 1–12.
- de Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56: 879–886.
- Delia J, Bravo-Valencia L, Warkentin KM. 2017. Patterns of parental care in Neotropical glassfrogs: fieldwork alters hypotheses of sex-role evolution. *Journal of Evolutionary Biology* 30: 898–914.
- Duellman WE, Trueb L. 1994. *Biology of Amphibians*. Johns Hopkins University Press, Baltimore, Maryland, USA. 670 p.
- Duellman WE. 1999. *Patterns of Distribution of Amphibians: A Global Perspective*. Johns Hopkins University Press, Baltimore, Maryland, USA. 633 p.
- Escalona-Sulbarán M, Simões PI, Gonzalez-Voyer A, Castroviejo-Fisher S. 2018. Neotropical frogs and mating songs: the evolution of advertisement calls in glassfrogs. *Journal of Evolutionary Biology* 32(2): 163–176.
- Graves GR. 1988. Linearity of geographic range and its possible effect on the population structure of Andean birds. *Auk* 105(1): 47–52.
- Guayasamin JM, Bonaccorso E. 2004. A new species of glass frog (Centrolenidae: *Cochranella*) from the lowlands of northwestern Ecuador, with comments on the *Cochranella granulosa* group. *Herpetologica* 60: 485–494.
- Guayasamin JM, Bustamante MR, Almeida-Reinoso D, Funk WC. 2006. Glass frogs (Centrolenidae) of Yanayaku Biological Station, Ecuador, with the description of a new species and comments on centrolenid systematics. *Zoological Journal of the Linnean Society* 147: 489–513.
- Guayasamin JM, Castroviejo-Fisher S, Ayarzagüena J, Trueb L, Vilá C. 2008. Phylogenetic relationships of glassfrogs (Centrolenidae) based on mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* 48: 574–595.
- Guayasamin JM, Castroviejo-Fisher S, Trueb L, Ayarzagüena J, Rada M, Vilá C. 2009. Phylogenetic systematics of glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. *Zootaxa* 2100: 1–97.
- Guayasamin JM, Cisneros-Heredia DF, Maynard R, Lynch RL, Culebras J, Hamilton PS. 2017. A marvelous new glassfrog (Centrolenidae, *Hyalinobatrachium*) from Amazonian Ecuador. *ZooKeys* 673: 1–20.
- Guayasamin JM, Cisneros-Heredia DF, Vieira J, Kohn S, Gavilanes G, Lynch RL, Hamilton PS, Maynard RJ. 2019. A new glassfrog (Centrolenidae) from the Chocó-Andean Río Manduriacu Reserve, Ecuador, endangered by mining. *PeerJ* 7: e6400.
- Hutter CR, Guayasamin JM. 2012. A new cryptic species of glassfrog (Centrolenidae: *Nymphargus*) from Reserva Las Gralarias, Ecuador. *Zootaxa* 3257: 1–21.
- Kalyaanamoorthy S, Minh BQ, Wong TK, von Haeseler A, Jermini LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589.
- Köhler J, Jansen M, Rodríguez A, Kok PJR, Toledo LF, Emmrich M, Glaw F, Haddad CFB, Rödel MO, Vences M. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods, and recommenda-

- tions for best practice. *Zootaxa* 4251(1): 1–124.
- Kubicki B, Salazar S, Puschendorf R. 2015. A new species of glassfrog, genus *Hyalinobatrachium* (Anura: Centrolenidae), from the Caribbean foothills of Costa Rica. *Zootaxa* 3920(1): 69–84.
- Lynch JD, Duellman WE. 1973. A review of the Centrolenid frogs of Ecuador, with descriptions of new species. *The University of Kansas Museum of Natural History Occasional Papers* 16: 1–66.
- Maddison DR, Maddison WP (2005) *MacClade 4: Analysis of Phylogeny and Character Evolution*. Sinauer Associates, Sunderland, Massachusetts, USA.
- McCracken SF, Forstner MRJ, Dixon JR. 2007. A new species of the *Eleutherodactylus lacrimosus* assemblage (Anura, Brachycephalidae) from the lowland rainforest canopy of Yasuni National Park, Amazonian Ecuador. *Phyllomedusa* 6(1): 21–33.
- Minh BQ, Nguyen MAT, von Haeseler A. 2013. Ultra-fast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 30: 1,188–1,195.
- Narins PM, Capranica RR. 1976. Sexual differences in the auditory system of the tree frog *Eleutherodactylus coqui*. *Science* 192: 378–380.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2014. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32: 268–274.
- de Oliveira EA, Hernández-Ruz EJ. 2017. New species of glassfrog, genus *Hyalinobatrachium* (Anura: Centrolenidae), for the Brazilian Amazon revealed by mitochondrial DNA and morphology. *International Journal of Research Studies in Biosciences* 5: 1–12.
- Padial JM, Castroviejo-Fisher S, Köhler J, Vilà C, Chaparro JC, De la Riva I. 2009. Deciphering the products of evolution at the species level: the need for an integrative taxonomy. *Zoologica Scripta* 38: 431–447.
- Polato NR, Gill BA, Shah AA, Gray MM, Casner KL, Barthelet A, Messer PW, Simmons MP, Guayasamin JM, Encalada AC, et al. 2018. Narrow thermal tolerance and low dispersal drive higher speciation in tropical mountains. *Proceedings of the National Academy of Sciences of the United States of America* 115(49): 12,471–12,476.
- Prum RO. 2017. *The Evolution of Beauty: How Darwin's Forgotten Theory of Mate Choice Shapes the Animal World—and Us*. Anchor Books, New York, New York, USA. 428 p.
- Ruiz-Carranza PM, Lynch JD. 1991. Ranas Centrolenidae de Colombia I. Propuesta de una nueva clasificación genérica. *Lozania* 57: 1–30.
- Ruiz-Carranza PM, Lynch JD. 1998. Ranas Centrolenidae de Colombia XI. Nuevas especies de ranas de cristal del género *Hyalinobatrachium*. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 22: 571–586.
- Safran RJ, Scordato ESC, Symes LB, Rodríguez RL, Mendelson TC. 2013. Contributions of natural and sexual selection to the evolution of premating reproductive isolation: a research agenda. *Trends in Ecology and Evolution* 28: 643–650.
- Shah AA, Funk WC, Ghalebabor CK. 2017. Thermal acclimation ability varies in temperate and tropical aquatic insects from different elevations. *Integrative and Comparative Biology* 57: 977–987.
- Salgado AL, Guayasamin JM. 2018. Parental care and reproductive behavior of the minute dappled glassfrog (Centrolenidae: *Centrolene peristictum*). *South American Journal of Herpetology* 13(3): 211–219.
- Savage JM, Heyer WR. 1967. Variation and distribution in the tree-frog genus *Phyllomedusa* in Costa Rica, Central America. *Beiträge zur Neotropischen Fauna* 5: 111–131.
- Simpson GG. 1951. The species concept. *Evolution* 5: 285–298.
- Simpson GG. 1961. *Principles of Animal Taxonomy*. Columbia University Press: New York, New York, USA. 247 p.
- Twomey EM, Delia J, Castroviejo-Fisher S. 2014. A review of northern Peruvian glassfrogs (Centrolenidae), with the description of four new remarkable species. *Zootaxa* 3851: 1–87.
- Wells KD. 2007. *The Ecology and Behavior of Amphibians*. University of Chicago Press, Chicago, Illinois, USA. 1,148 p.
- Wilczynski W, Ryan MJ. 1999. Geographic variation in animal communication systems. Pp. 234–261 In *Geographic Variation in Behavior*. Editors, Foster SA, Endler JA. Oxford University Press, New York, New York, USA. 314 p.
- Wiley EO. 1978. The evolutionary species concept reconsidered. *Systematic Zoology* 27: 17–26.
- Zakon HH, Wilczynski W. 1988. The physiology of the anuran eighth nerve. Pp. 125–155 In: *The Evolution of the Amphibian Auditory System*. Editors, Frittsch B, Ryan MJ, Wilczynski W, Hetherington TE, Walkowiak W. John Wiley and Sons, New York, New York, USA. 705 p.

Appendix 1. Examined Specimens

Hyalinobatrachium esmeralda: Colombia: *Boyacá Department*: Municipio de Pajarito, Inspección Policía Corinto, finca 'El Descanso', quebrada 'La Limonita', 1,600–1,650 m, ICN 9592–94, 9596, 9602–03 (type series of *H. esmeralda*).

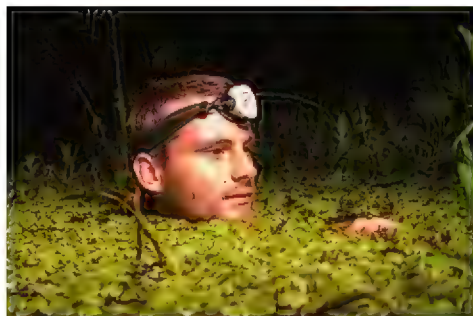
Hyalinobatrachium pellucidum: Ecuador: *Morona Santiago Province*: Nueva Alianza, Finca Santa Catalina (78.1335°W, 2.100°S; 1,305 m), Límite del Parque Nacional Sangay, MEPN 14706. Quebrada del Río Napinaza (78.4070°W, 2.9266°S, 1,100 m), QCAZ 42000; km 6.6 on the Limón-Macas road (ca. 2.92816°S, 78.344°W; 1,013 m), QCAZ 29438; 6 km N of Limon, QCAZ 25950. *Sucumbíos Province*: Río Azuela (0.1167°S, 77.6167°W; 1,740 m), Quito-Lago Agrio road; KU 164691 (holotype), USNM 286708–10; Río Reventador, USNM 286711–12. *Zamora Chinchipe Province*: Cordillera del Cóndor, Miazhi Alto (4.25044°S, 78.61356°W; 1,282 m), QCAZ 41560–61, 41648.

Hyalinobatrachium munozorum: Ecuador: *Sucumbíos Province*: Santa Cecilia (00°03'N, 76°58'W; 340 m), KU 118054 (holotype), 105251, 123225, 150620 (paratypes), 152488–89, 155493–96, 175504. *Orellana Province*: Tiputini Biodiversity Station, ZSFQ DFCH-USFQ D105. Colombia: *Meta Department*: Meta, ICN 5031–34, 39503. *Amazonas Department*: Leticia, ICN (field number JMR 4119).

Hyalinobatrachium yaku: Ecuador: *Pastaza Province*: stream affluent of the Kallana river (1.4696°S, 77.2784°W; 325 m), MZUTI 5001 (holotype), 5002 (paratype). *Orellana Province*: Timburi-Cocha Research Station (0.4800°S, 77.2829°W; 300 m) near San José de Payamino, QCAZ 55628, QCAZ 53352, 53354, 56664. *Napo Province*: Ahuano (1.0632°S, 77.5265°W; 360 m), ZSFQ 02322.



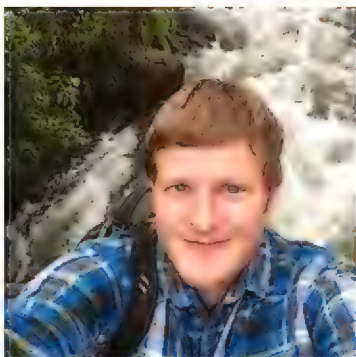
Juan M. Guayasamin is a professor at Universidad San Francisco de Quito, Ecuador, and codirector of the Laboratory of Evolutionary Biology. Juan obtained his Master's and Ph.D. degrees in ecology and evolutionary biology from the University of Kansas (Lawrence, Kansas, USA) under the supervision of Dr. Linda Trueb. He is member of the Ecuadorian Academy of Sciences and has published more than 80 scientific papers on evolution, systematics, biogeography, and conservation of Neotropical animals, mainly amphibians.



Jose Vieira is a field biologist, wildlife photographer, and tour leader from Venezuela. From a young age, Jose became passionate about nature, particularly amphibians and reptiles. This passion led him to participate in countless field expeditions in his native country, and from them Jose has contributed many herpetological specimens to the Museo de Historia Natural La Salle. Currently, his contributions to science continue in Ecuador with the rediscovery of the critically endangered *Atelopus bomolochos* and *A. nepiozomus*, and his expeditions to remote areas of the country to work on various herpetological projects of Tropical Herping and Universidad San Francisco de Quito.



Richard E. Glor is a Professor in the Department of Ecology and Evolutionary Biology, and a Curator in the Biodiversity Institute, at the University of Kansas (Lawrence, Kansas, USA). Richard studies the evolution of species diversity, primarily through work on West Indian anole lizards. He received his Bachelor's degree from Cornell University (Ithaca, New York, USA), his doctorate in Ecology and Evolutionary Biology from Washington University (St. Louis, Missouri, USA), and conducted postdoctoral research in the Center for Population Biology at University of California, Davis.



Carl R. Hutter recently obtained his Ph.D. from the University of Kansas (Lawrence, Kansas, USA). Carl is interested in amphibians and has done field research in Madagascar and Ecuador. He is currently focusing on the evolution of advertisement calls in frogs, especially seeking to understand how environmental influences lead to the evolution of distinct calls. Carl is also interested in the phylogenomics of frogs, and is working to understand anuran phylogenetic relationships at the Order level, as well as within several families.



New records and distribution extension of the rare glassfrog *Hyalinobatrachium chirripoi* (Anura: Centrolenidae) throughout the Chocó-Magdalena region in Colombia

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Hyalinobatrachium is the most diverse glassfrog genus (family Centrolenidae) with 32 species described to date, ranging from Mexico to Argentina (Guayasamin et al. 2009; Frost 2019). Given the low levels of morphological differentiation within this genus, species identification is sometimes difficult, and requires the use of alternative sources of evidence such as molecular phylogenetics and DNA barcoding (Castroviejo-Fisher et al. 2009). *Hyalinobatrachium chirripoi* (Taylor 1958) is a seldom observed species found in forests under 600 m elevation from Honduras, along the Chocó-Darien to the Esmeraldas Province in north Ecuador (Kubicki 2007; Guayasamin et al. 2016). Here new records of *H. chirripoi* are reported which extend the distribution of this species into the Andean foothills of the central Chocó and, for the first time, into the Magdalena Valley of Colombia. An overview of the known distribution *H. chirripoi* is presented, including previous museum records and the new data.

Specimens examined. One individual was collected in 2010 at Vereda El Porton, in San Francisco, Antioquia, Colombia (5.9015, -74.96925, 589 m asl; Fig. 1), in the Magdalena River drainage. The individual was found at night, calling on the underside of a *Heliconia* leaf overhanging a small stream in a secondary forest, and was euthanized with an overdose of 2% Roxicaine and fixed in 10% formalin. A liver sample was preserved in 99% ethanol. The specimen was deposited in the Museo de Herpetología, Universidad de Antioquia, Colombia

(voucher MHUA-A 06650; originally misidentified as *H. fleischmanni*). In 2016, two other individuals were collected during nocturnal surveys performed at 20 km SW of Condoto, Chocó, Colombia, on the western versant of the Cordillera Occidental (5.02078, -76.51633, 423 m asl; Fig. 1A). They were found calling from the upper side of leaves in a tree hanging above a small river (Fig. 1B). They were captured and euthanized with an overdose of topical lidocaine hydrochloride (Xylocaine). Muscle samples were stored in 100% ethanol. Specimens were then fixed with 100% ethanol and deposited in the herpetology collection of the Natural History Museum at Universidad de los Andes, Colombia (vouchers ANDES-A3738 and ANDES-A3739). Other individuals at the same locality were observed on leaves and fronds of Araceae, Musaceae (*Heliconia*), and ferns (Polypodiaceae), perched ~3–8 m off the ground. Both localities (Vereda El Porton at the Magdalena River drainage, and 20 km SW of Condoto in the Chocó) are classified as tropical wet forest biome (bh-T, Holdridge 1964).

Morphological and molecular identification. These samples were identified as *H. chirripoi* based on light dorsal spots, significant webbing between Fingers II and III, clear parietal peritoneum, bare heart condition (i.e., iridophores covering all visceral peritonea except for the urinary bladder and pericardium), tympanum visible, and a truncate snout in sagittal view (Taylor 1958; Ruiz-Carranza and Lynch 1998; Savage 2002, Fig. 2). To

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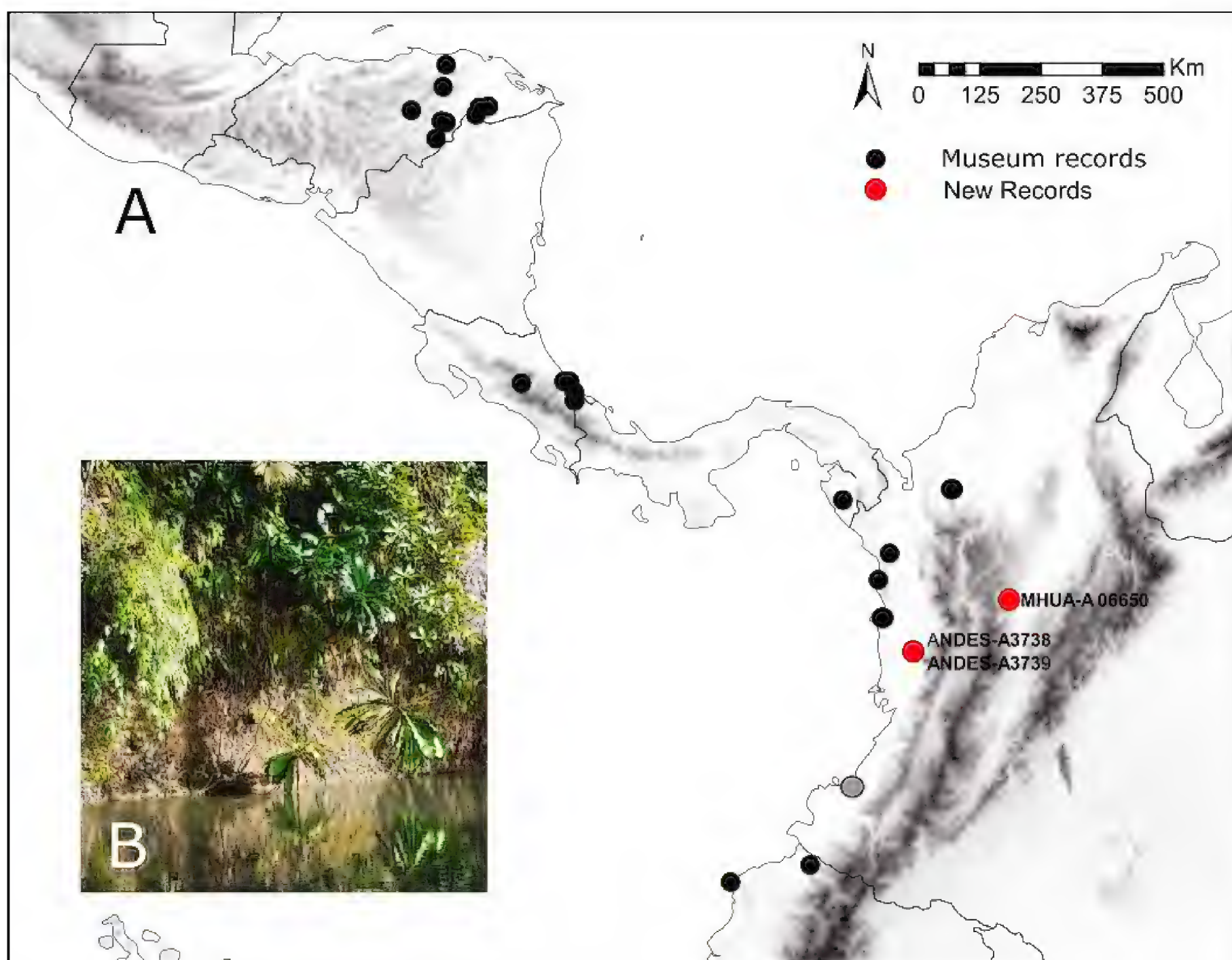


Fig. 1. (A) Localities of museum specimens (black dots) and new records (red dots) for *Hyalinobatrachium chirripoi*. Coordinates for specimen IAvH-A-4311 (gray dot) were approximated to the urban area of Río Guapi, Cauca, since precise coordinates of the collection site were lacking. (B) Habitat where the ANDES-A individuals were encountered.

further corroborate morphological diagnoses, mtDNA barcoding was used. DNA was extracted following Ivanova et al. (2006) for specimens ANDES-A3738 and ANDES-A3739, or using the Thermo Scientific DNA extraction kit for specimen MHUA-A 06650. Amplification of 16S (567 bp) and COI (609 bp) loci was as described by Guayasamin et al. (2008), and Mendoza et al. (2016; primers from Meyer et al. 2005), respectively. Purified products were Sanger-sequenced in both directions. Sequences obtained are deposited in GenBank under accession numbers MH129045–49.

Sequences of both genes were blasted against the GenBank non-redundant database using megaBLAST. COI sequences were also used as input for the BOLD DNA barcoding system (Ratnasingham and Hebert 2007). In addition, Kimura-two-parameter (K2P; Kimura 1980) pairwise distances between sequences of closely related *Hyalinobatrachium* species available in GenBank (Table 1) were calculated using MEGA7 (Kumar et al. 2016) and maximum likelihood and Bayesian mtDNA genealogies were built with RAxML v.8.2.10 (Stamatakis 2006, 2014), and MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003, Ronquist et al. 2012), respectively.

Maximum likelihood searches used the rapid hill-

climbing algorithm and 10,000 rapid bootstrap pseudo-replicates to assess nodal support. In MrBayes two independent 2,000,000 generation analyses were run, sampling every 1,000 generations, and with 20% burn-in. The best models for molecular evolution for the 16S and for each codon position of the COI gene were selected using PartitionFinder 2 (Lanfear et al. 2016).

Mitochondrial sequences unambiguously confirmed the identity of the specimens as *H. chirripoi*. All BLAST searches against GenBank returned *H. chirripoi* sequences as the top hit, with 99% identity and e-values of zero. Online BOLD identification searches matched the COI sequences to *H. chirripoi* with 99.3–99.5% identity. On the other hand, *H. fleischmanni* sequences matched the query sequences with 83.8% similarity (BOLD) and 83.6% identity (GenBank). Maximum likelihood and Bayesian trees corroborated these results, with the query sequences nested within a well-supported clade that includes all the other *H. chirripoi* (Fig. 3). Finally, K2P distances among *H. chirripoi* samples averaged 0.006 (range = 0.002–0.009) for 16S and 0.027 (0–0.039) for COI, while the mean distance with *H. colymbiophyllum*, its sister species, was 0.021 (0.018–0.024) for 16S and 0.081 (0.059–0.093) for COI.

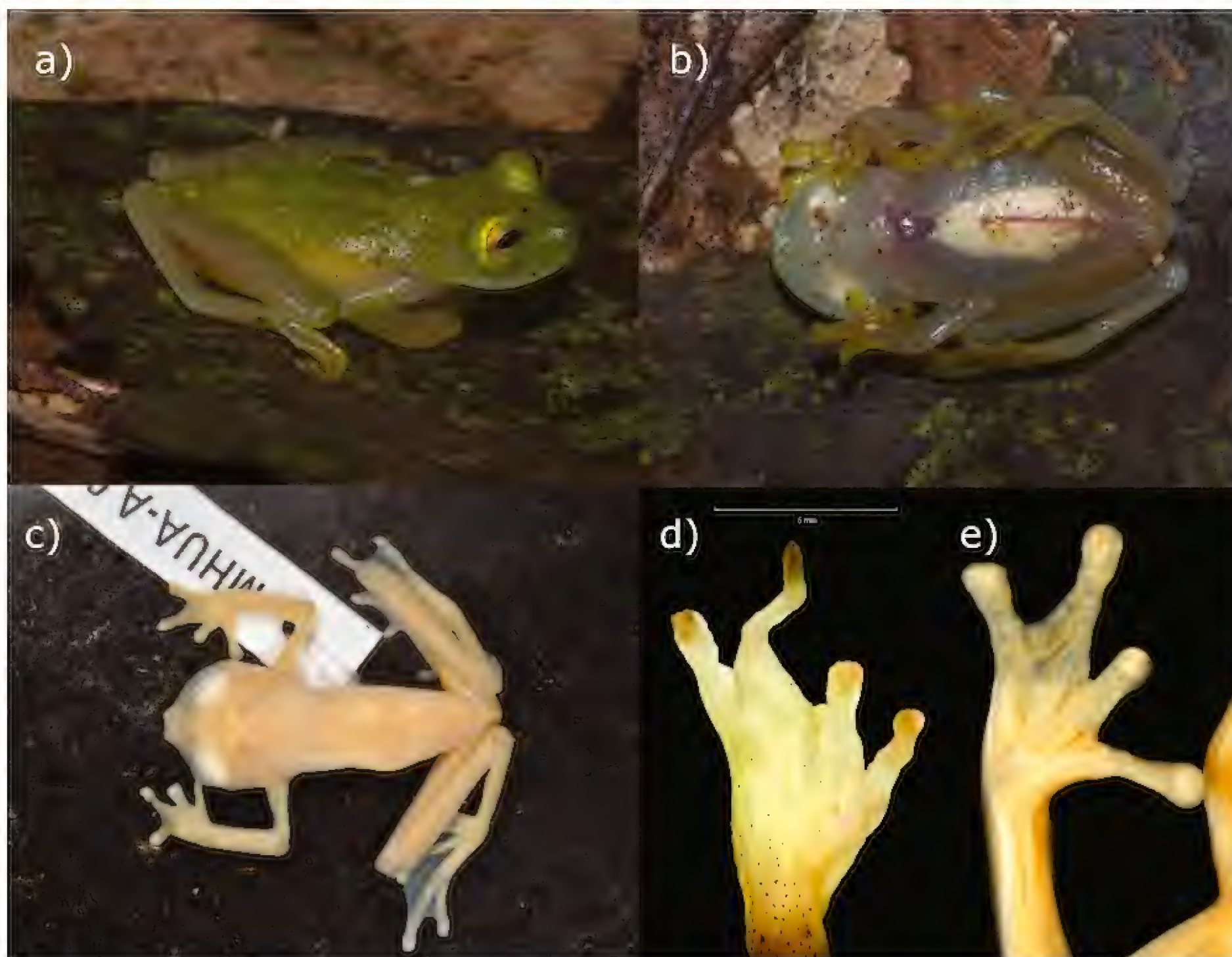


Fig. 2. Dorsal (a) and ventral (b) view of live specimen from Chocó-Darien (ANDES-A-3738). Dorsal view (c) of specimen collected in Magdalena basin (MHUA-A-6650). Details of hand webbing of specimens collected in Chocó-Darien (d) and Magdalena basin (e).

Distribution and conservation implications. The records of *H. chirripoi* since its rediscovery by Kubicki (2004) are very scarce. In Colombia there have been very few isolated records of the species (Hayes and Starret 1980; Romero-Martínez et al. 2008; Ruiz-Carranza and Lynch 1998). Most previous records for the species in Colombia are restricted to the Northwest Chocó-Darien region close to Panama, in Nuquí (MHUA-A 5150-53), and in Bahía Solano (ICN 40270-314) (Fig. 1). One additional specimen was collected in 1987 further south, near Río Guapi, Cauca (specimen IAvH-Am-4311) with no georeferenced locality (gray circle in Fig. 1) and the southernmost specimens were collected from Esmeraldas Provinces in Ecuador (QCAZ-A 48271, QCAZ-A 66603, Guayasamin et al. 2016). The new records reported here fill the gap in the Chocó-Darien between the Bahía Solano and Río Guapi records, extending the distribution of this species 70 km into the Chocoan mainland and into the foothills of the Western Andes (400 m asl).

These records also extend the distribution of *H. chirripoi* into the Magdalena basin, across the Andes from all previous records of this species. Previously, the closest record of *H. chirripoi* to the Magdalena basin was from the Cerro Murrucucú in Tierralta, Córdoba,

within the Parque Nacional Natural Paramillo (ICN 39129–30), an intermediate zone between Chocó-Darien rainforests and Magdalena basin. This region is included in the Sinú-San Jorge District, characterized by a biota with common elements, including several amphibian species of the Chocoan, Amazonian, and Magdalenian regions (Henao-Sarmiento et al. 2008; Hernández-Camacho et al. 1992a; Marquez et al. 2017; Romero-Martínez et al. 2008; Vasquez and Serrano 2009), and is considered as a transition zone between the Chocó-Darien, Caribbean, and Magdalena bioregions (Romero-Martínez et al. 2008). Congruently, the Magdalena basin record reported here lies within the Nechí District, for which the biological elements have affinity with those from the upper Sinú and high San Jorge drainages, as well as the Chocó-Darien region (Hernández-Camacho et al. 1992b).

With these new records of *H. chirripoi*, the Magdalena basin and Chocó-Darién regions in Colombia share a total of five species of the genus (including *H. fleischmanni*, *H. colymbiphylum*, *H. aureoguttatum*, and *H. valerioi*). *Hyalinobatrachium aureoguttatum* and *H. valerioi* are easily differentiable by the dorsal coloration (large yellow round spots on a green background), but

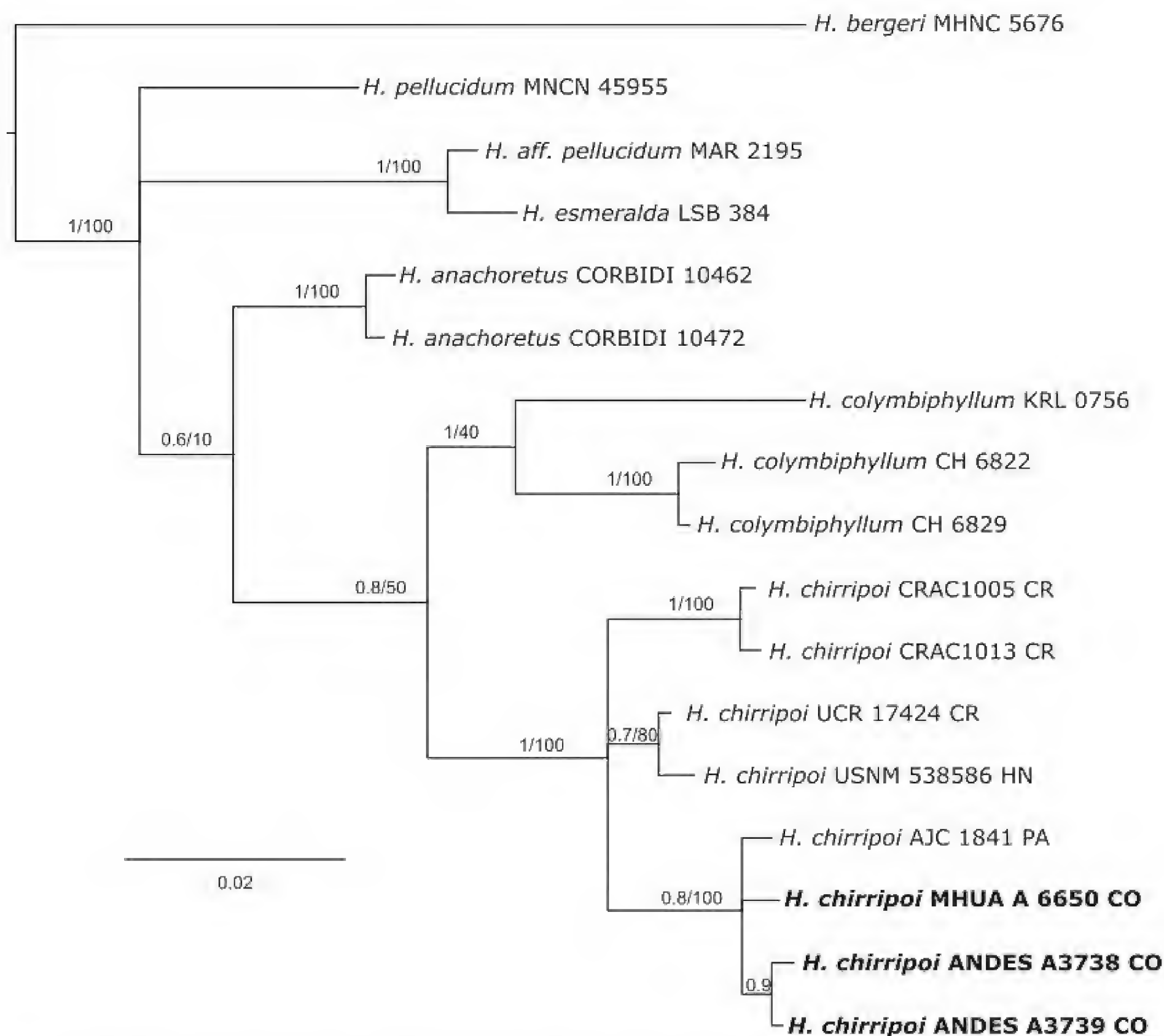


Fig. 3. Phylogenetic positions of three *Hyalinobatrachium chirripoi* samples from Chocó and Antioquia, Colombia, in a Bayesian mtDNA tree inferred from 16S rRNA and COI sequences. The chosen models of evolution using Partition finder were: 16S: GTR+I, COI position 1: GTR+I, position 2: SYM+G, and position 3: F81+I. Samples in bold are from this study, while the others are from GenBank. Posterior probability and bootstrap support values (from a maximum likelihood analysis) are indicated in front of the corresponding node as PP/Bootstrap. Two letter country codes provided for *H. chirripoi* samples follow the International Organization for Standardization: CO = Colombia, PA = Panama, HN=Honduras, CR = Costa Rica.

misidentification is common for the other three species (Kubicki 2004). The most relevant external feature for differentiating *H. chirripoi* is the extensive webbing between Fingers II–III (*H. colymbiphyllum* and *H. fleischmanni* have little webbing between Fingers II–III); additionally *H. fleischmanni* has iridophores covering the pericardium, while *H. chirripoi* and *H. colymbiphyllum* lack iridophores in the pericardial peritonea (Savage 2002; Starret and Savage 1973, but check Cisneros-Heredia and McDiarmid 2007). After a detailed revision of the *H. fleischmanni* specimens for the Magdalena basin stored in the Museo de Herpetología of Universidad de Antioquia, no additional misidentified *H. chirripoi* were found. However, this work highlights the importance of carefully inspecting museum specimens of *Hyalinobatrachium* (and other taxa with

low morphological differentiation between species) when using such specimens for biogeographic and conservation work, in order to avoid errors associated with misidentification.

A shortage of information still remains on the amphibian diversity in Chocó-Darien rainforest and Magdalena basin, both of which are increasingly threatened by human activities such as mining, habitat loss, fragmentation, and other forms of landscape transformation (Etter and van Wyngaarden 2000; Rangel 2004). Indeed, according to the IUCN Red List, certain populations of *H. chirripoi* in Panama and Colombia are threatened by habitat loss, due to increasing agricultural activity and logging (Solís et al. 2008). The new records presented here provide additional information about the distribution of this rare species, and highlight the

Table 1. Sequences for mitochondrial regions 16S and COI of *Hyalinobatrachium chirripoi* and related species used in this study.

Species	Voucher	16S	COI
<i>H. chirripoi</i>	ANDES-A3738	MH129045	MH129047
<i>H. chirripoi</i>	ANDES-A3739	MH129046	MH129048
<i>H. chirripoi</i>	MHUA-A-6640	MH129049	NA
<i>H. chirripoi</i>	UCR 17424	EU663037	NA
<i>H. chirripoi</i>	USNM 538586	EU663038	NA
<i>H. chirripoi</i>	AJC 1841	KF604299	KF604294
<i>H. chirripoi</i>	CRAC1005	NA	KJ703104
<i>H. chirripoi</i>	CRAC1013	NA	KJ703105
<i>H. bergeri</i>	MHNC 5676	EU663033	NA
<i>H. pellucidum</i>	MNCN 45955	KM068262	NA
<i>H. esmeralda</i>	LSB 384	KP149361	KP149161
<i>H. colymbiphyllum</i>	KRL 0756	FJ784359	NA
<i>H. colymbiphyllum</i>	CH 6829	KR863254	KR862999
<i>H. colymbiphyllum</i>	CH 6822	KR863256	KR863001
<i>H. anachoretus</i>	CORBIDI 10462	KM068268	NA
<i>H. anachoretus</i>	CORBIDI 10472	KM068300	NA
<i>H. aff. pellucidum</i>	MAR-2195	KM068296	NA

importance of using an integrative taxonomic approach at the junction between these two bioregions in terms of biodiversity conservation, as well as the need for continued documentation of their biological richness.

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Agudelo-Zamora provided the images from specimens deposited at Instituto de Ciencias Naturales, Facultad de Ciencias, Universidad Nacional de Colombia. We thank Celsa Señaris and Jesse Delia for their invaluable comments to earlier versions of this manuscript, and Juan M. Daza (Universidad de Antioquia, Colombia) for access to Museo de Herpetología Universidad de Antioquia (MHUA) and his invaluable support in the development of this manuscript.

Literature Cited

Castroviejo-Fisher S, Vilá C, Ayarzagüena J, Blanc M, Ernst R. 2011. Species diversity of *Hyalinobatrachium* glassfrogs (Amphibia: Centrolenidae) from the Guiana Shield, with the description of two new species. *Zootaxa* 3132: 1–55.

Cisneros-Heredia DF, McDiarmid RW. 2007. Primer registro de *Hyalinobatrachium ruedai* (Amphibia: Centrolenidae) en Ecuador, con notas sobre otras especies congénéricas. *Herpetotrópicos* 3: 21–28.

Etter A, Wyngaarden W. 2000. Patterns of landscape transformation in Colombia, with emphasis in the Andean region. *AMBIO: A Journal of the Human Environment* 29: 432–439.

Frost DR. 2019. Amphibian Species of the World: an Online Reference. Version 6.0. Available: <http://research.amnh.org/herpetology/amphibia/index.html> [Accessed: 28 July 2019].

Guayasamin JM, Castroviejo-Fisher S, Ayarzagüena J, Trueb L, Vilá C. 2008. Molecular phylogenetics and evolutionary phylogenetic relationships of glassfrogs (Centrolenidae) based on mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* 48: 574–595.

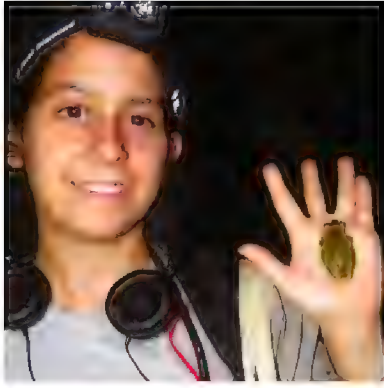
Guayasamin JM, Castroviejo-Fisher S, Trueb L, Ayarzagüena J, Rada M, Vilá C. 2009. Phylogenetic systematics of glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. *Zootaxa* 2100: 1–97.

Guayasamin JM, Varela-Jaramillo A, Frenkel C, Pazmiño-Armijos G. 2016. *Hyalinobatrachium chirripoi*. Unpaginated In: *Anfibios del Ecuador*. Version 2018.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador. Available; <https://bioweb.bio/faunaweb/amphibiaweb/FichaEspecie/Hyalinobatrachium%20chirripoi> [Accessed: 20 November 2018].

Hayes MP, Starrett PH. 1980. Notes on a collection of centrolenid frogs from the Colombian Chocó. *Bulletin of the Southern California Academy of Sciences* 79: 89–96.

Henao-Sarmiento JE, Cárdenas-Torres MA, Fajardo-Patiño A. 2008. Zonificación ambiental de la zona de reserva forestal del Pacífico en jurisdicción del departamento de Córdoba, Caribe Colombiano. *Colombia Forestal* 11: 175–200.

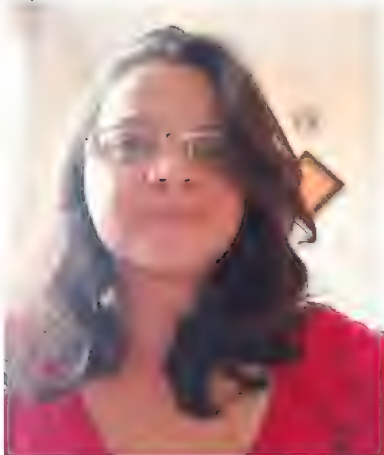
- Hernández-Camacho J, Hurtado-Guerra A, Ortiz-Quijano R, Walschburger T. 1992a. Centros de endemismo en Colombia. Pp. 175–190 In: *La Diversidad Biológica de Iberoamérica I*. Acta Zoológica Mexicana. CYTED-B, Instituto de Ecología, Xalapa, Veracruz, México. 389 p.
- Hernández-Camacho J, Hurtado-Guerra A, Ortiz-Quijano R, Walschburger T. 1992b. Unidades biogeográficas de Colombia. Pp. 105–152 In: *La Diversidad Biológica de Iberoamérica I*. Acta Zoológica Mexicana. CYTED-B, Instituto de Ecología, Xalapa, Veracruz, México. 389 p.
- Holdridge L. 1964. *Life Zone Ecology*. Tropical Science Center, San José, Costa Rica. 206 p.
- Ivanova NV, deWaard JR, Hebert PDN. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* 6: 998–1,002.
- Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120.
- Kubicki B. 2004. Rediscovery of *Hyalinobatrachium chirripoi* (Anura: Centrolenidae) in southeastern Costa Rica. *Revista de Biología Tropical* 52: 215–218.
- Kubicki B. 2007. *Ranas de Vidrio Costa Rica/Glass Frogs of Costa Rica*. Instituto Nacional de Biodiversidad, INBio, Santo Domingo de Heredia, Costa Rica. 312 p.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1,870–1,874.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773.
- Marquez R, Mejia-Vargas D, Palacios-Rodriguez P, Ramirez-Castaneda V, Amezcuita A. 2017. A new species of *Andinobates* (Anura: Dendrobatidae) from the Urabá region of Colombia. *Zootaxa* 4290: 531–546.
- Mendoza ÁM, Torres MF, Paz A, Trujillo-Arias N, López-Alvarez D, Sierra S, Forero F, Gonzalez MA. 2016. Cryptic diversity revealed by DNA barcoding in Colombian illegally traded bird species. *Molecular Ecology Resources* 16: 862–873.
- Meyer CP, Geller JB, Paulay G. 2005. Fine scale endemism on coral reefs: archipelagic differentiation in turbinid gastropods. *Evolution* 59: 113–125.
- Rangel CJO. 2004. *Colombia Diversidad Biotica IV. El Choco Biogeografico/Costa Pacifica*. Universidad Nacional de Colombia, Bogotá, Colombia. 997 p.
- Ratnasingham S, Hebert PD. 2007. BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Resources* 7: 355–364.
- Romero-Martínez HJ, Vidal-Pastrana CC, Lynch JD, Dueñas PR. 2008. Preliminary study of the amphibian fauna of the Cerro Murrucucú, Parque Natural Nacional Paramillo, Tierralta, Córdoba, Colombia. *Caldasia* 30: 209–229.
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Ruiz-Carranza PM, Lynch JD. 1998. Ranas Centrolenidae de Colombia XI. Nuevas especies de ranas cristal del género *Hyalinobatrachium*. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 22: 571–586.
- Savage JM. 2002. *The Amphibians and Reptiles of Costa Rica: a Herpetofauna between Two Continents, between Two Seas*. University of Chicago Press, Chicago, Illinois, USA. 954 p.
- Solís F, Ibáñez R, Chaves G, Savage J, Jaramillo C, Fuenmayor Q, Castro F, Grant T, Wild E, Kubick B. 2008. *Hyalinobatrachium chirripoi*. The IUCN Red List of Threatened Species 2008: e.T55006A11235906. Available: <http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T55006A11235906.en> [Accessed: 14 December 2017].
- Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2,688–2,690.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1,312–1,313.
- Starrett PH, Savage JM. 1973. The systematic status and distribution of Costa Rican glass-frogs, genus *Centrolenella* (family Centrolenidae), with description of a new species. *Bulletin of the Southern California Academy of Sciences* 72: 57–78.
- Taylor EH. 1958. Notes on Costa Rican Centrolenidae with descriptions of new forms. *University of Kansas Science Bulletin* 39: 41–68.
- Vásquez VH, Serrano MA. 2009. *Las Áreas Naturales Protegidas de Colombia*. Conservación Internacional Colombia y Fundación Biocolombia, Bogotá, Colombia. 696 p.



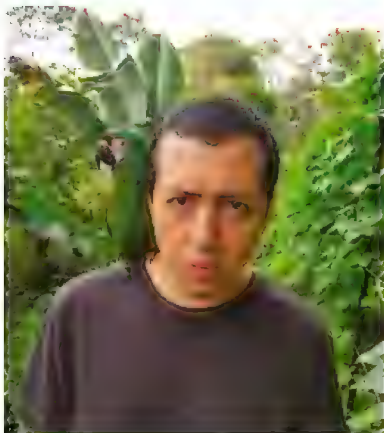
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Natural history notes on three sympatric frogs, *Amolops formosus* (Günther 1875), *Nanorana liebigii* (Günther 1860), and *Ombrana sikimensis* (Jerdon 1870), from Manaslu Conservation Area, Nepal

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Abstract.—Three stream dwelling mountain frogs, *Amolops formosus*, *Nanorana liebigii*, and *Ombrana sikimensis* are sympatric species, native to Asia and distributed much across Nepal. Here, a brief natural history account of the three species is provided that enhances the existing knowledge of these understudied frogs. Altogether 21 adults (eight *Amolops formosus*, six *Nanorana liebigii*, and seven *Ombrana sikimensis*) were collected from the streams of Sirdibas, Chumchet, and Bihi villages in April and May 2016 and in March 2017. Since the survey time coincided with breeding season, egg clutches and tadpoles of *Nanorana liebigii* were observed. Basic morphometric features of the adults (snout-vent length, head length, head width, femur length, and tibia length) and tadpoles (total length, body length, body width, and tail muscle width) were measured with a Mitutoyo digital Vernier caliper to the nearest 0.1 mm. Environmental parameters of the habitat were also noted, including air temperature, water temperature, relative humidity, and pH of the water body. A review of the conservation status of these sympatric frogs highlights the threats they face from unchecked harvesting in Manaslu and across the entire mountain villages of Nepal. Other potential threats include declining stream habitats through water use management decisions such as dams and diversions, pollution, and forest degradation. The field observation data collected will help to fill in the knowledge gaps for these species, in order to prioritize conservation action and aid future research.

Keywords. Amphibia, Anura, Asia, habitat degradation, morphometrics, threats

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Introduction

Amolops formosus, *Nanorana liebigii*, and *Ombrana sikimensis* are sympatric species that are largely dependent upon mountain brooks and associated riparian habitats characterized by coniferous or oak forests (Schleich and Kästle 2002). They are native to Asia, found across many of the mountains of Nepal, and also recorded in India, China, Bangladesh, and Bhutan (Bordoloi et al. 2004; Liang et al. 2004). In Nepal, they are distributed within an altitudinal range of 1,190–3,360 m asl (Schleich and Kästle 2002). All three species were previously placed in the genus *Rana* (Boulenger 1920), but later revised into distinctive genera of *Amolops*, *Nanorana*, and *Ombrana* (Chen et al. 2005; Dubois 1974; Frost et al. 2006). The earliest first-hand records related to morphometrics, life history, and habitat notes of the three species (Boulenger 1882; Günther 1860; Jerdon 1870)

are not readily accessible at the present time. While the recent publication of Schleich and Kästle (2002) is rather comprehensive, it is still unavailable to many readers due to the high price of the book (Zug 2004). Shah and Tiwari (2004) provided little information on the associated habitats of these sympatric amphibians, like surrounding vegetation and environmental parameters, but their report lacks data on egg deposition and tadpole stages. The IUCN Red List Assessment 2004 has further emphasized the need for research on the taxonomy, population size, distribution, trends, ecology, and life history of these frogs to prioritize conservation actions (Bordoloi et al. 2004; Liang et al. 2004). Therefore, any readily-available publication on the natural history of these frogs is of great importance to the scientific community, conservationists, natural resource managers, and decision makers.

Stream-dwelling frogs serve as good indicators of the stream ecosystem health, since they are philopatric

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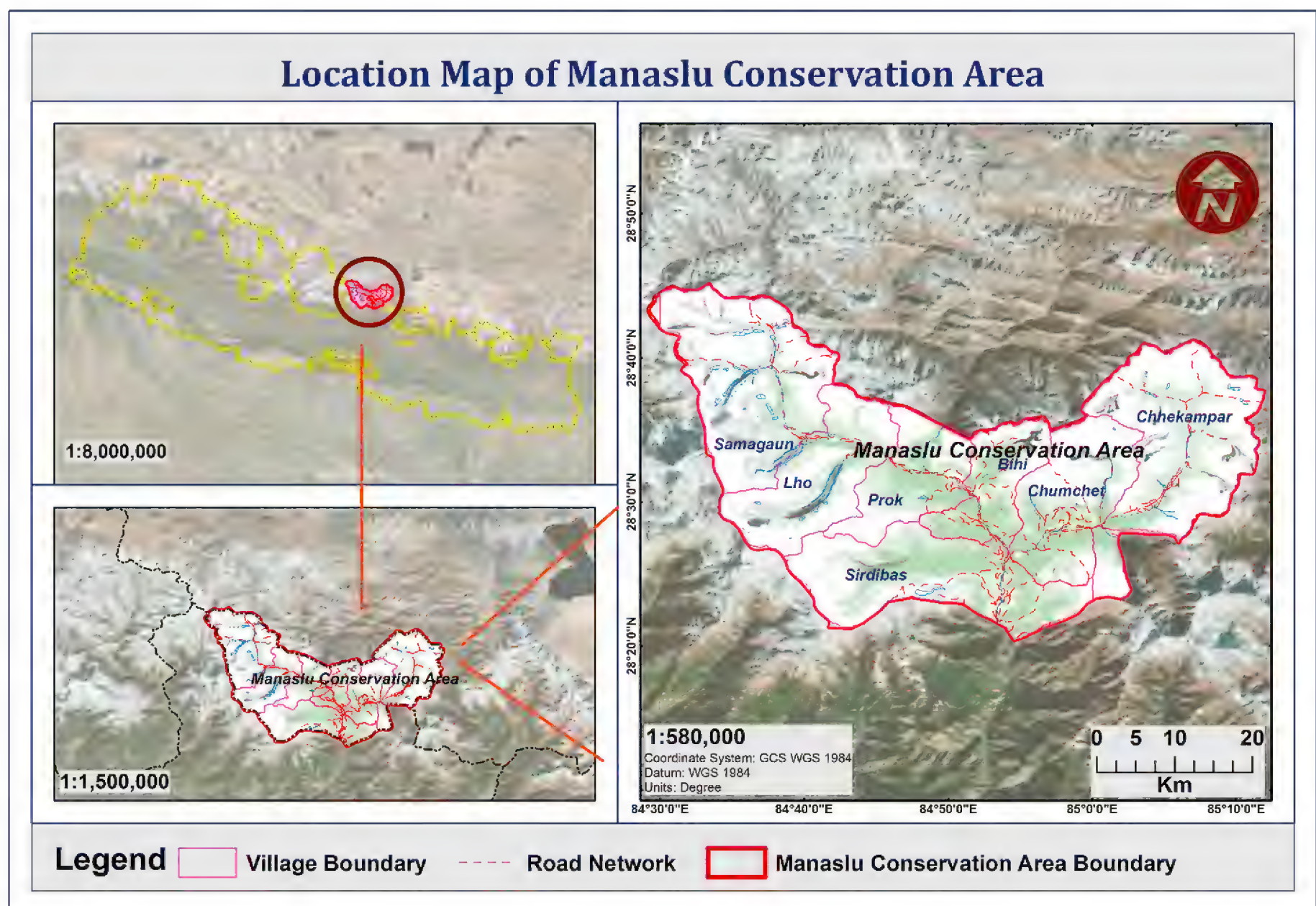


Fig. 1. Study sites in Manaslu Conservation Area, Nepal, with villages indicated in blue text.

in nature and found in steady populations (Welsh and Ollivier 1998). Studying such stream frogs with respect to morphology, life history, and habitat conditions will help to further understanding of their ecological niches (Ningombam 2009). This knowledge is vital for devising efficient conservation strategies when one-third of the total amphibian species of the world are being threatened with extinction (Baillie et al. 2004). This study presents the natural history notes of these three sympatric frogs. This information will help to aid in further research and monitoring, while providing background support for good decision making regarding their conservation in the future.

Materials and Methods

Surveys. Manaslu Conservation Area is one of the protected areas in Nepal, located at the upper north area of Gorkha district, province number 4 (Gandaki Pradesh). Surveys were conducted in five major villages of the Manaslu Conservation Area, namely Sirdibas, Bihi, Chumchet, Prok, and Samagaun, excluding Lho and Chhekampar (Fig. 1). The entire survey spanned 49 days during the day-time in April–May 2016 and March 2017.

A distance of 279 km was covered on foot throughout the survey and a transect of 200 m was walked in each site of the 14 streams (Table 1). Time-constrained searches were conducted for 2 h with two people at a time, for a total of four person-h per search. Live specimens of *Amolops formosus*, *Nanorana liebigii*, and *Omrana sikimensis* were collected for morphological

examination. They were released in-situ after recording observational notes and taking photographs with a Canon EOS 700D (18–135 mm) kit lens DSLR camera (Fig. 2). Egg clutches and tadpoles of different sizes were found in a few streams, and they were closely observed while causing minimal disturbance. The distribution of these sympatric frogs in Manaslu is restricted to Sirdibas, Bihi, and Chumchet (Fig. 3).

Measurements. The snout-vent length (SVL), head length (HL), head width (HW), femur length (FL), and tibia length (TL) were the morphological parameters measured following Fei et al. (2009) for the adult frogs. The morphometric keys for tadpoles were total length (TL), body length (BL), body width (BW), and tail muscle width (TMW), and followed Mitchell et al. (2012). All the measurements were taken using a Mitutoyo digital Vernier caliper to the nearest 0.1 mm.

Air and water temperature measurements were taken using a digital thermometer. The humidity was measured using a Hygrometer and the pH of the water was recorded with a digital pH meter. Geographic coordinates and altitude were recorded with a Garmin eTrex 10 GPS. Species identification and additional information followed Boulenger (1882, 1920), Chen et al. (2005), Frost et al. (2006), Günther (1860), Ningombam (2009), Schleich and Kästle (2002), and Shah and Tiwari (2004).

Results and Discussion

Morphometrics. The morphological notes of the three sympatric frogs correspond well with the earlier

Three sympatric frogs in Manaslu Conservation Area, Nepal

Table 1. Sampling locations and observations of frogs (adults, egg clutches, and tadpoles) in the survey.

Sampling Site	Village	Location	Altitude (m asl)	Survey Time	Observations
1	Sirdibas	Yuwang Khola*	1,622	Day	<i>Amolops formosus</i> (2 ♀, 1 ♂); <i>Nanorana liebigii</i> (5 tadpoles, egg clutch)
2	Sirdibas	Ghatte Khola	2,425	Day	None
3	Sirdibas	Myarchwang Khola	1,629	Day	<i>Nanorana liebigii</i> (1 ♂); <i>Ombrana sikimensis</i> (7 individuals, unidentified sex)
4	Chumchet	Gyanak Khola	2,294	Dawn	<i>Amolops formosus</i> (3 ♀); <i>Nanorana liebigii</i> (1 ♂)
5	Chumchet	Sipchet Ripchet	2,473	Day	<i>Nanorana liebigii</i> (1 ♂)
6	Chumchet	Chumling	2,485	Dawn	<i>Nanorana liebigii</i> (1 ♀)
7	Chumchet	Gumlung Khola	2,482	Day	None
8	Chumchet	Sardi Khola	1,938	Dawn	<i>Nanorana liebigii</i> (tadpole with metamorphosed legs)
9	Chumchet	Lokpa	1,887	Day	<i>Nanorana liebigii</i> (egg clutch)
10	Chumchet	Phujung Khola	1,931	Day	<i>Nanorana liebigii</i> (1 ♂)
11	Samagaun	Birendra Tal	3,700	Day	None
12	Prok	Namrung Khola	2,462	Day	None
13	Bihi	Bihi Khola	2,189	Day	<i>Amolops formosus</i> (1 ♀); <i>Nanorana liebigii</i> (1 ♂)
14	Bihi	Dyang Khola	1,838	Night	<i>Amolops formosus</i> (1 ♀)

*Khola refers to stream

descriptions provided by Günther (1860, 1875) and Jerdon (1870) quoted in Schleich and Kästle (2002). *Nanorana liebigii* has the largest mean body size and body weight followed by *Ombrana sikimensis* and *Amolops formosus*. The body weight measurements of these frogs found in Nepal did not set new records. In Bhutan, Wangchuk (2017) documented the average weight of *Nanorana liebigii* as exceptionally higher (males 500–750 g and females 350–500 g) than the present findings for Nepal. The head lengths (HL) of *Amolops formosus* and *Nanorana liebigii* were smaller than the head widths (HW), however, *Ombrana sikimensis* was different, with HL greater than HW (Table 2). On the contrary, Boulenger (1920) has described broader HW than HL in *Ombrana sikimensis*.

Frogs in general tend to exhibit sexual dimorphism, with females mostly being larger in body size than males (Monnet and Cherry 2002). The results here agree for *Amolops formosus*, where females were larger in size than males; however, the males of *Nanorana liebigii* were

larger in size than their female counterparts. The adult males of *Amolops formosus* had a nuptial pad on the 1st finger of the forelimb and also partly turquoise-colored hind limbs on the ventral side (Fig. 4A), as documented by Schleich and Kästle (2002). Likewise, males of the *Nanorana liebigii* had strongly hypertrophied forelimbs with a nuptial pad on the 1st finger. Further, numerous black horny spines were present on the 1st, 2nd, and 3rd fingers on both the arms and extending along the pectoral region (Fig. 4C). It was difficult to identify the sexes of *Ombrana sikimensis* based only on SVL measurements, since nuptial spines are not present in *Ombrana sikimensis* (Boulenger 1920).

Egg deposition and larval stages. Spring (March–May) is the season of breeding for *Nanorana liebigii* as egg clutches were found in slowly drifting Yuwang Khola in Sirdibas village to fast flowing streams in Lokpa, Chumchet village. The eggs were attached to the undersides of stones, totally submerged, and white in

Table 2. Morphological parameters (SVL, HL, HW, FL, and TL) of the three species of adult sympatric frogs (mm) and BW (g). Min = Minimum value, Max = Maximum value, M = Average value (Mean), SD = Standard Deviation, and n = number of individuals.

Morphometric keys	<i>Amolops formosus</i> (n = 8)			<i>Nanorana liebigii</i> (n = 6)			<i>Ombrana sikimensis</i> (n = 7)		
	Min	M ± SD	Max	Min	M ± SD	Max	Min	M ± SD	Max
Snout-vent length (SVL)	67.3	74.1 ± 3.8	81.5	78.8	87.7 ± 7.6	99.6	67.1	81.2 ± 11.6	92.1
Head length (HL)	23.7	24.7 ± 0.7	26.1	25.8	27.9 ± 2.0	31.5	21.6	24.6 ± 2.3	27.1
Head width (HW)	24.6	25.8 ± 1.0	27.9	27.4	29.1 ± 1.8	32.4	20.5	23.3 ± 2.2	25.8
Femur length (FL)	39.9	42.9 ± 1.9	46.1	43.2	50.6 ± 3.7	53.1	39.3	44.0 ± 3.9	47.9
Tibia length (TL)	45.2	47.5 ± 1.2	49.1	47.6	54.7 ± 3.9	58.7	42.3	46.4 ± 3.4	50.2
Body weight (BW)	35	46.9 ± 6.2	55	60	82.8 ± 14.8	100	50	70.1 ± 14.6	85



Fig. 2. Dorsal view of live adults: (A) *Ombrana sikimensis*, (B) *Amolops formosus*, and (C) *Nanorana liebigii*. (D) Dorso-lateral view of *Nanorana liebigii*. Photos: Biraj Shrestha and Min Bahadur Gurung.

color inside the gelatinous ball that had a honeycomb-like appearance (Fig. 5A–B). The clutch size consisted of about 80–140 eggs although no adults were seen nearby, which is noteworthy since males of *Nanorana liebigii* are reported to guard the eggs as a form of parental care (Rai 2003). Some gradually developing egg clutches were observed that had embryos with eyes and were surrounded by jelly of a “liver-like” color (Fig. 5C).

Five tadpoles of *Nanorana liebigii* were observed at the Yuwang Khola and one metamorphosed tadpole with hind limbs was evident in the shallow pools of a rapidly flowing stream in Sardi Khola. The metamorphosed tadpole had a well-developed oral disc (Fig. 5D), with the structure of the upper and lower lips forming an atrium feature (Kästle et al. 2013). The tails of the tadpoles were nearly twice the length of the body (Schleich and Kästle 2002), while the body lengths were significantly greater than widths (Table 3).

Tadpoles of *Nanorana liebigii* were found sympatric with the tadpoles of *Duttaphrynus himlayanus*. However, no egg clutches of *Amolops formosus* were observed during the study. Published information on egg deposition by *Amolops formosus* is limited, though Nidup et al. (2016) reported an egg clutch of *Amolops himlayanus* attached underneath of rocks and clear white, from a gentle flowing stream in Bhutan.

Habitat notes. The general habitat of all the three sympatric frogs studied here is mountain streams above 1,100 m asl

elevation and of varied intensity. In addition, *Nanorana liebigii* was also found to inhabit other water bodies, such as the puddles in a bamboo forest and irrigation ditches near the cropland where *Karu* (a type of naked barley) was grown (Fig. 6). *Amolops formosus* preferred fast flowing streams, typically cascades, attaching themselves to the steep slopes of rocks and resting on fissures, partly covered in moss and ferns, while *Ombrana sikimensis* were typically hiding in clusters underneath rocks in shallow streams (Fig. 6C). The nearby riparian vegetation included Nepalese Alder (*Alnus nepalensis*), Broom Grass (*Thysanolaena maxima*), Himalayan Blue Bamboo (*Himalayacalamus hookerianus*), Himalayan Silver Birch (*Betula utilis*), Tree Rhododendron (*Rhododendron arboreum*), Chir Pine (*Pinus roxburghii*), Walnut (*Juglans*

Table 3. Morphological parameters (TL, BL, BW, and TMW) of the tadpoles of *Nanorana liebigii* (mm). Min = Minimum value, Max = Maximum value, M = Average value (Mean), SD = Standard Deviation, and n = number of individuals.

Morphometric keys	<i>Nanorana liebigii</i> Tadpole (n = 6)		
	Min	M ± SD	Max
Tail length (TL)	41.4	49.1 ± 6.7	57.7
Body length (BL)	13.3	19.3 ± 4.5	24.5
Body width (BW)	8.3	11.6 ± 2.5	14.7
Tail muscle width (TMW)	3.2	7.3 ± 3.3	11.5

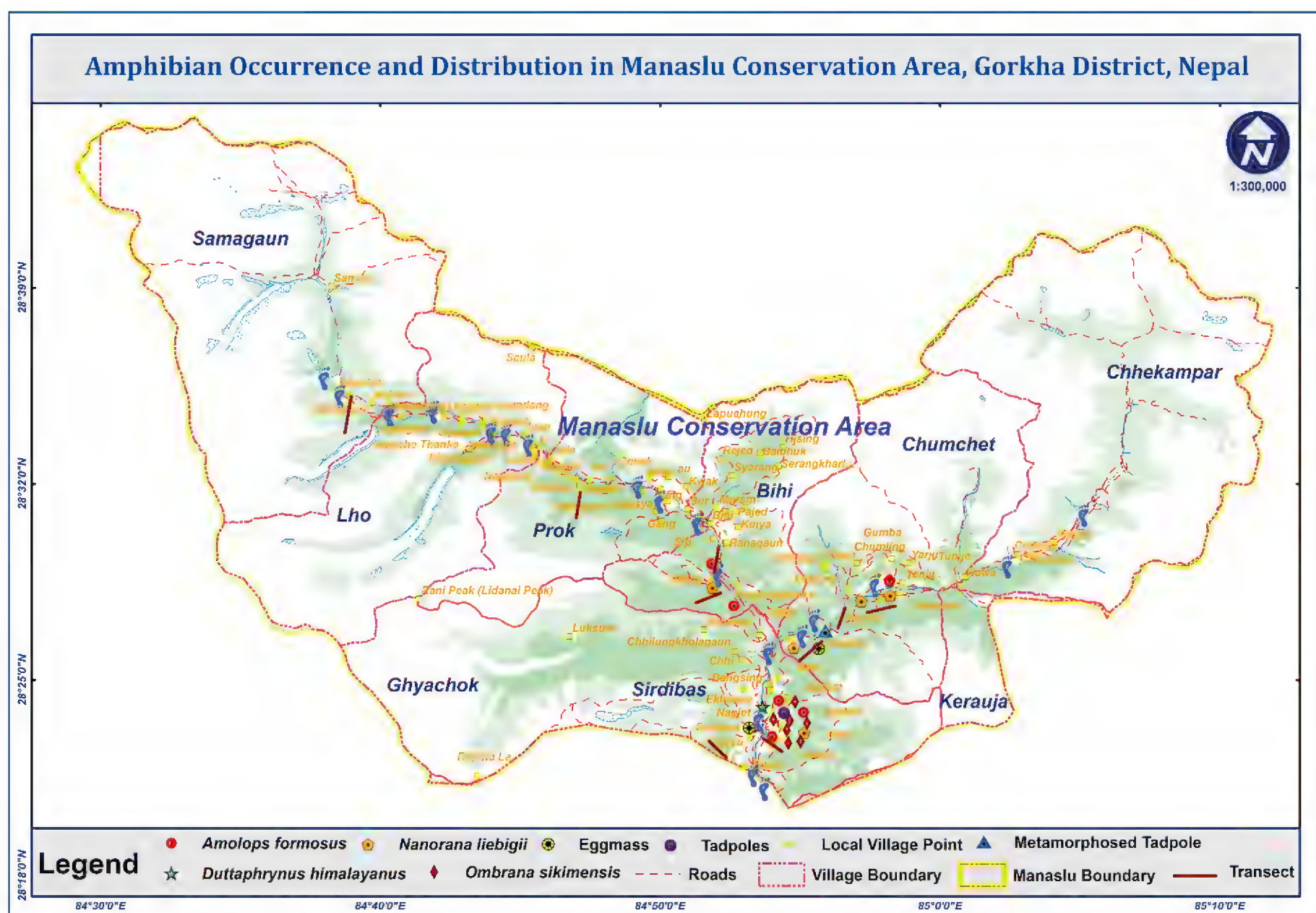


Fig. 3. Frog observation sites and distances travelled throughout the survey.

regia), and others.

Amphibians are often mentioned as the bio-indicators of water quality because of their permeable skin. Their eggs and larval stages are generally much more vulnerable to any type of pollution in the water bodies due to their lack of protective covering, and direct connection with the water for survival and growth. The optimal environmental conditions of their habitat are crucial to allow for metamorphosis and for habitat management strategies. Cold water is favorable for the growth of different life stages with neutral to slightly alkaline water pH. This range is desirable for much of the aquatic fauna, including stream dwelling frogs, as lower acidic pH conditions can impede amphibian growth and inhibit the development of eggs and embryos (Ningombam 2009).

Conservation status. All the three species of frogs are listed in the Least Concern (LC) category in the IUCN Red List Assessment from 2004 (15 years prior to this writing) based upon the presumption of their large populations, wider distributions, and with no prospects of immediate decline. However, a reassessment is desperately needed, as the current population trends for all three species are going down due to declining stream habitats from various causes, such as water diversion and dams to deforestation and pollution (Bordoloi et al. 2004; Liang et al. 2004). In India, all three of these species are protected under the national legislation, while no similar effort by the Nepalese government has been undertaken to provide any legal measures for amphibian conservation. This remains the case today (in 2019), despite the recommendation by the Biodiversity Profiles

Project (BPP, 1995) for nine species of endemic Nepalese amphibians to be included in the Schedule I of National Parks and Wildlife Conservation (NPWC) Act 1973.

Anurans of genera *Amolops*, *Nanorana*, and *Ombrana* are called ‘Paha’ frogs in Nepal and have ethnozoological relationships with the communities living mostly in hills and mountains (Shah and Tiwari 2004; Shrestha 2018). People often harvest paha frogs as a delicacy and for their apparent therapeutic benefits. Every indigenous community in the mountains of Nepal either has experience in paha hunting or at least knows about its use. As a result, paha hunting is popular in villages from the east to western part, all across the nation. The hunting usually takes place at night during pre- and post-monsoon seasons, when the water flow is minimum. There is no limit for harvested quantities from the streams, and people usually collect as many as they can find during their searches. In Manaslu, Gurung communities in Sirdibas village typically collect 51–100 individuals on

Table 4. Physico-chemical characteristics of water quality in the survey sites and altitudinal range of the detected frogs. Min = Minimum value, Max = Maximum value, M = Average value (Mean), and SD = Standard Deviation.

Abiotic factors	Min	M ± SD	Max
Air Temperature (°C)	8	20.0 ± 6.5	26.5
Water Temperature (°C)	4	13.2 ± 2.8	16.3
Relative Humidity (%)	25	43.5 ± 11.7	55
pH	7	8.0 ± 0.3	8.6
Altitude (m asl)	1,591	1,880.5 ± 39.4	2,480



Fig. 4. Ventral views of live adults: (A) male of *Amolops formosus*, (B) female of *Amolops formosus*, (C) male of *Nanorana liebigii*, and (D) female of *Nanorana liebigii*. Photos: Biraj Shrestha.

average in one season and they trade locally in the price range of USD 0.45–2.26 (Shrestha and Gurung 2019). *Nanorana liebigii* is the most popular paha frog across the country, followed by *Ombrana sikimensis* which is highly sought after as its meat serves as a delicacy and nutritional purposes (Shah and Tiwari 2004). In addition, the meats of *Nanorana liebigii* and *Amolops formosus* are traditionally assumed to have medicinal properties that cure fever, cough, cold, dysentery, and stomach ache; while their skin secretions have antiseptic properties (Shrestha and Gurung 2019). In recent years, paha hunting is largely practiced to enjoy its meat and for recreational purposes in the villages, since the nutritional requirements are often met by poultry and livestock, and medical supplies are readily available thanks to improved road access for most villages these days. However, the continued paha hunting practice has depleted its numbers as reported by the local communities across the country, including Manaslu, and has led to the recommendation for some form of legal conservation protection.

The diminishing populations of *Amolops formosus*, *Nanorana liebigii*, and *Ombrana sikimensis* can be averted by developing species specific conservation priority plans. Habitat conservation planning, population study and monitoring, hunting regulation policies, and effective outreach programs are some of the key action steps. The brief natural history notes presented here on morphometrics, sexual dimorphism, egg deposition, larval stages, and habitat conditions will be helpful in this regard. But since the identification of congeneric amphibians can be tricky, the use of molecular phylogeny

and call identification coupled with morphometrics is strongly recommended for accurate species identification.

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Literature Cited

- Baillie JEM, Hilton TC, Stuart SN. 2004. *IUCN Red List of Threatened Species. A Global Species Assessment*. International Union for Conservation of Nature, Gland, Switzerland and Cambridge, United Kingdom. 191 p.
- Bordoloi S, Ohler A, Shrestha TK. 2004. *Ombrana sikimensis*. *The IUCN Red List of Threatened Species* 2004: e.T58246A11757068.



Fig. 5. Life stages of *Nanorana liebigii*: (A) eggs deposition underneath a stone, (B) egg clutch, (C) embryo development, and (D) tadpole with metamorphosed legs. Photos: Biraj Shrestha.



Fig. 6. Habitat varieties of the three sympatric frogs: (A) rapidly flowing stream, (B) series of waterfalls inhabited by *Amolops formosus*, (C) slow flowing shallow stream, and (D) irrigation ditch. Photos: Biraj Shrestha.

- Bordoloi S, Ohler A, Shrestha TK, Ahmed MF. 2004. *Amolops formosus*. *The IUCN Red List of Threatened Species* 2004: e.T58206A11746654.
- Boulenger GA. 1882. *Catalogue of the Batrachia Salientia s. Ecaudata in the Collection of the British Museum*. Second Edition. Taylor and Francis, London, United Kingdom. 503 p., 30 pls.
- Boulenger GA. 1920. *A Monograph of the South Asian, Papuan, Melanesian and Australian Frogs of the Genus Rana*. Records of the Indian Museum, Volume 20. Zoological Survey of India, Calcutta, India. 226 p.
- BPP [Biodiversity Profiles Project]. 1995. *Red Data Book of the Fauna of Nepal Biodiversity*. Profiles Project Technical Publication No. 4. Department of National Parks and Wildlife Conservation, Ministry of Forests and Soil Conservation, His Majesty's Government of Nepal, Kathmandu, Nepal.
- Chen L, Murphy RW, Lathrop A, Ngo A, Orlov NL, Ho T, Somorjai ILM. 2005. Taxonomic chaos in Asian ranid frogs: An initial phylogenetic resolution. *Herpetological Journal* 15: 231–243.
- Dubois A. 1974. Liste commentée d'amphibiens récoltés au Nepal. *Bulletin du Museum National d'Histoire Naturelle* 213: 341–411.
- Fei L, Hu SQ, Ye CY, Huang YZ. 2009. *Fauna Sinica: Amphibia*. Science Press, Beijing, China. 957 p.
- Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, de Sá RO, Channing A, Wilkinson M, Donnellan SC, et al. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* 297: 1–291.
- Günther ACLG. 1860. Contribution to the knowledge of the reptiles of the Himalaya Mountains. *Proceedings of the Zoological Society of London* 1860: 148–175.
- Jerdon TC. 1870. Notes on Indian herpetology. *Proceedings of the Asiatic Society of Bengal* 1870: 66–85.
- Kästle W, Rai K, Schleich H. 2013. *Field Guide to Amphibians and Reptiles of Nepal*. ARCO-Nepal, Munich, Germany. 609 p.
- Liang F, Lau MWN, Dutta S, Shrestha TK, Borah MM. 2004. *Nanorana liebigii*. *The IUCN Red List of Threatened Species* 2004, e.T58428A11780058.
- Mitchell T, Alton LA, White CR, Franklin CE. 2012. Relations between conspecific density and effects of ultraviolet-B radiation on tadpole size in the Striped Marsh Frog: UVBR and conspecific-density effects on amphibians. *Conservation Biology* 26(6): 1,112–1,120.
- Monnet JM, Cherry MI. 2002. Sexual size dimorphism in anurans. *Proceedings of the Royal Society of London B Biological Sciences*. 269: 2,301–2,307
- Nidup T, Gyeltshen D, Dorji S, Pearch MJ. 2016. The first record of *Amolops himalayanus* (Anura: Ranidae) from Bhutan. *The Herpetological Bulletin* 136: 13–18.
- Ningombam B. 2009. Amphibian fauna in and around Loktak Lake, Manipur, India with reference to the genus *Amolops* Günther. Ph.D. Dissertation, Gauhati University, Jalukbari, Guwahati, Assam, India.
- Rai KR. 2003. Environmental impacts, systematics, and distribution of herpetofauna from east Nepal. Ph.D. Dissertation, Tribhuvan University, Kirtipur, Kathmandu, Nepal.
- Schleich H, Kästle W. 2002. *Amphibians and Reptiles of Nepal: Biology, Systematics, Field Guide*. First Edition. A.R.G. Ganter Verlag, Ruggell, Liechtenstein. 1,201 p.
- Shah KB, Tiwari S. 2004. *Herpetofauna of Nepal: A Conservation Companion*. IUCN Nepal, Kathmandu, Nepal. 237 p.
- Shrestha B. 2018. *Amphibian Conservation: Brief Introduction in the Context of Nepal*. The Rufford Foundation, London, United Kingdom; SAVE THE FROGS!, Laguna Beach, California, USA; and Resources Himalaya Foundation, Kathmandu, Nepal. 20 p.
- Shrestha B, Gurung MB. 2019. Ethnoherpetological notes regarding the Paha Frogs and conservation implications in Manaslu Conservation Area, Gorkha District, Nepal. *Journal of Ethnobiology and Ethnomedicine* 15(1): 23.
- Wangchuk S. 2017. Morphometric Study of Mon-Paa Frog: A Case Study of Dopuchen, Dumtoe and Tendruk Gewog under Samtse Dzongkhag. Dzongkhag Forestry Sector, Samtse Dzongkhag, Bhutan. 24 p.
- Welsh HH, Ollivier LM. 1998. Stream amphibians as indicators of ecosystem stress: A case study from California's redwoods. *Ecological Applications* 8(4): 1,118–1,132.
- Zug GR. 2004. Book Review: Amphibians and Reptiles of Nepal: Biology, Systematics, Field Guide. *Herpetological Review* 35(1): 88–90.



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Checklist of the amphibians and reptiles of the Lely Mountains, eastern Suriname

Rawien Jairam

National Zoological Collection of Suriname, Anton de Kom University, Paramaribo, SURINAME

Abstract.—The Lely Mountains in Suriname have been surveyed only a few times by various herpetologists since 1973, and most recently in June 2016 by three people during six days. The total number of species recorded from the Lely Mountains is 102, including 46 species of amphibians and 41 species of reptiles in the 2016 surveys, with 15 additional species from the previous survey. Pluviometric conditions were not favorable during the survey, so more species of amphibians likely remained undetected. The use of only active searches probably only allowed detection of a portion of the reptile fauna of the massif. Unfavorable weather and the relatively small area sampled indicate that the diversity of the herpetofauna of the Lely Mountains is probably far from being completely documented. The presence of roads established for illegal gold mining and other human structures, such as a communication tower, have caused significant forest degradation.

Keywords. Anura, conservation, herpetofauna, Lacertilia, laterite landscape, new records, Serpentes, South America, Testudines

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Introduction

Presently the herpetofauna of Suriname consists of approximately 303 species (Ouboter 2017). Although over 90% of Suriname is still covered by natural vegetation, only a few studies have assessed the herpetofaunal diversity of Suriname throughout the entire territory (Hoogmoed 1973; Ouboter and Jairam 2012) leaving many areas still unexplored. Herpetofaunal surveys in specific areas have been conducted by several researchers (Ouboter et al. 2007, 2011; Nielsen et al. 2013; Fouquet et al. 2015a,b). New country records are still being documented, e.g., *Amapasaurus tetradactylus* (Jairam and Jairam-Doerga 2015), and range extensions continue to be reported, e.g., *Ptychoglossus brevifrontalis* (Jairam and Jairam-Doerga 2016).

The Lely Mountains are located in the eastern part of Suriname (4°25'–4°45'N, 54°39'–54°55'W) and together with the Brownsberg, Nassau, Winti Wai, Hoka-Hin, Stonbroekoe, and Majordam Mountains they form a system of laterite-bauxite plateaus in northeastern Suriname. These small plateaus form a unique type of landscape (H. ter Steege, pers. comm.) with different vegetation types, including high forest, savannah forest, and rocky creek beds (Alonso and Mol 2007). The Lely

Mountains together with the Brownsberg and the Nassau Mountains comprise a bauxite concession of the Suriname Aluminum company (Suralco), which formerly operated in Suriname (Mol et al. 2007). Although Suralco explored the Lely Mountains for bauxite deposits, the company did not proceed with the mining exploitation. The Lely Mountains consist of several plateaus with a maximum height of approximately 700 m (Alonso and Mol 2007). Due to the absence of established roads for cars, the Lely Mountains are mainly accessible only by air. A trail that is used by gold miners going up the Lely Mountains by means of all-terrain vehicles is long and tedious, and seldom without dangers. These circumstances have led to relatively few surveys (Hoogmoed 1974, 1975, unpub.; Watling and Ngadino 2007) at this location when compared to other nearby bauxite mountains, such as the Brownsberg Mountain. The remoteness of this location may also favor the occurrence of microendemics and/or rare species such those as in *Anomaloglossus* (Vacher et al. 2017). However, information about the herpetofauna of the Lely Mountains is still scant. This report documents new findings from the herpetofauna surveyed in the Lely Mountains in June 2016, and presents an overview of the herpetofaunal diversity observed and an updated list for this location.

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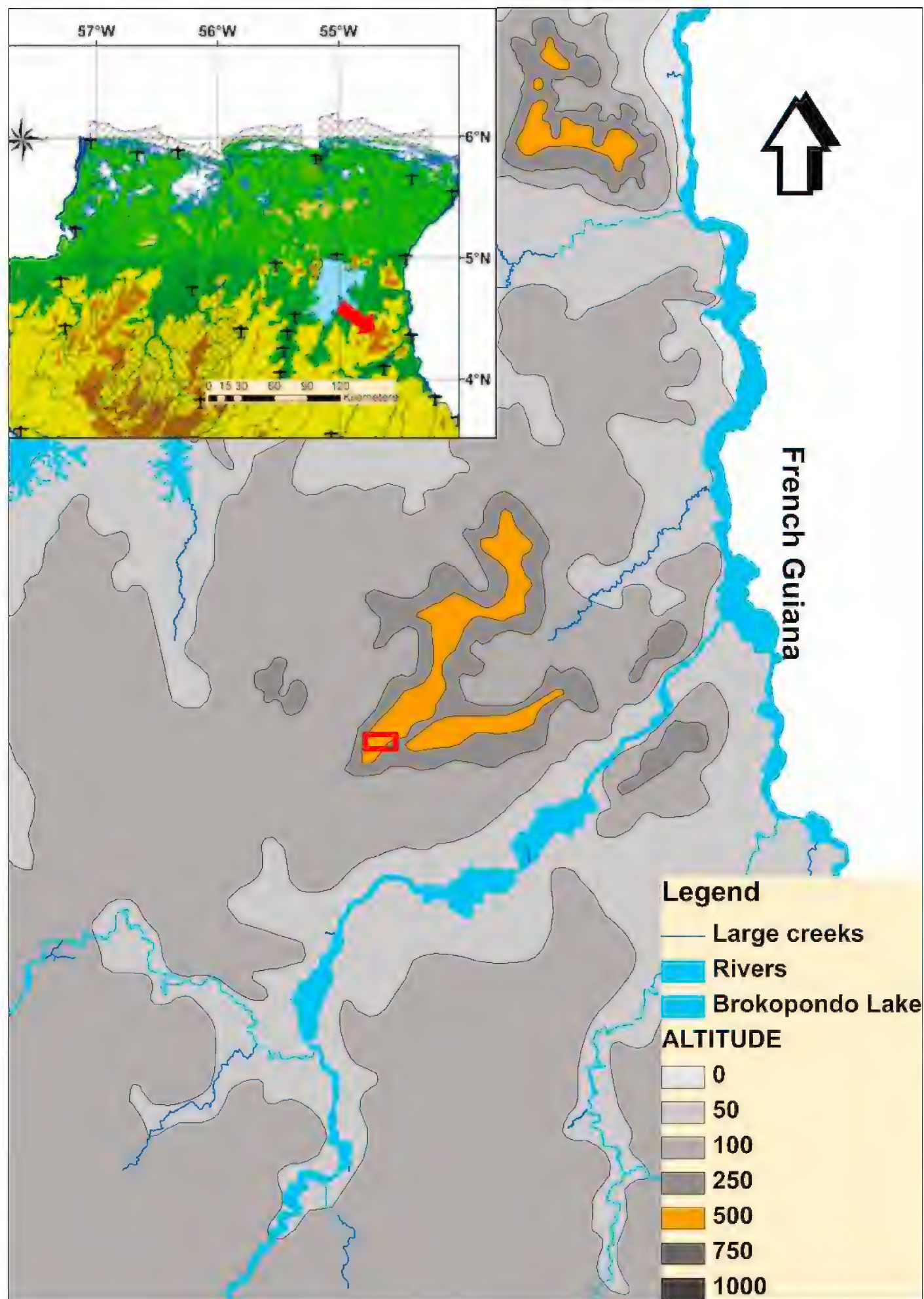


Fig. 1. Map of the Lely Mountains plateau showing the area surveyed in the red square. The inset image shows the location of the Lely Mountains in Suriname, as indicated by the red arrow. In the right upper side of the figure the Nassau Mountains are visible.

Materials and Methods

Habitat description. The vegetation and water drainages in the Lely Mountains are presently heavily disturbed locally by illegal small-scale gold miners. Some of the areas sampled were the airstrip, which was covered with grass, and small ditches located on one side for water drainage. The area around the airstrip was surrounded by patches of pristine forest interspersed with disturbed forests. The vegetation in the disturbed areas included mostly trees that were approximately two m high. The cause of this disturbance could have been the establishment of the airstrip by Suralco. The

bauxite company also established roads that extend from the plateau to the northern part of the Lely Mountains. The aforementioned roads were usually dominated by large water filled potholes bordered by small shrubs and bushes. Also sampled was a small creek on the plateau, which was heavily disturbed by artisanal gold mining.

Survey methods. Three people opportunistically surveyed the forest of the Lely Mountains using available roads and trails for six days. Trails and roads were surveyed radiating in different directions from the airstrip (Fig. 1). The surveyors also walked through the forest away from trails to increase the chance of finding amphibians and

reptiles. Night surveys started at dusk, at approximately 19.00 h, and usually lasted up until 24.00 h into the night. Amphibians and reptiles were actively searched for and were collected by hand when encountered. Call records were made for calling amphibians using a Marantz recorder with an external microphone. All recorded calls were analyzed using the software Audacity (<http://audacityteam.org>) and compared against currently known anuran call databases. Pools on the road and in other potential locations were checked for tadpoles. Searches for amphibians and reptiles were conducted during the day. All collected specimens were photographed, and afterwards were sedated and sacrificed in the field using Lidocaine®. Specimens were preserved in 10% formalin (one part full-strength formaldehyde and nine parts water) and after a few days they were transferred to 70% ethanol for storage at the National Zoological Collection of Suriname (NZCS). The identities of some of the amphibian specimens collected were verified by molecular analyses, as indicated in Table 1. Additionally, some data are provided below on the habitats where the species were collected, and whether they are documented here for the first time for the Lely Mountains.

Historical survey data. To complete the present list of species known from the Lely Mountains searches were performed in the online databases from Naturalis (<http://bioportal.naturalis.nl>) and GBIF (<https://www.gbif.org/species/search>) to check for species from this location that were not formally mentioned in the published literature. Figure 2 gives an overview of the total number of species documented in the three herpetological surveys at the Lely Mountains (Hoogmoed in 1974/5, Watling and Ngadino in 2006, and the fieldwork reported here in 2016).

Results

A total of 28 species of amphibians, representing seven families, and 18 species of reptiles in 11 families, were collected (Figs. 3–8). All specimens were unambiguously identified to the species level. Previously, 19 anuran species were recorded for the Lely Mountains (Watling and Ngadino 2007). The two lists combined yield a total of 29 species, eight of which were never before documented for the Lely Mountains. For the Lely Mountains, the RAP (Rapid Assessment Program) reported a total of 18 reptile species (Watling and Ngadino 2007); the present survey also collected a total of 18 species, seven of which were new records for this location. Database searches of Naturalis (<http://bioportal.naturalis.nl>) and GBIF (<https://www.gbif.org/species/search>) provided a total of 34 anuran species, 17 of which were not documented during either the RAP or the survey held in June 2016. For reptiles, Naturalis and GBIF yielded a total of 25 species, 13 of which were not documented in the surveys. Taking into account all of the surveys and database reports for the Lely Mountains yields a total of 46 anurans and 41 reptiles identified to the species level (Table 1).

Some noteworthy observations were made for some of the amphibian and reptile species collected in June 2016.

- *Anomaloglossus stepheni* was collected for the first time in the Lely Mountains. Specimens were collected in the leaf litter in a patch of dry pristine forest approximately one km north of the airstrip.
- *Boana xerophylla* is formally reported for the first time for the Lely Mountains, and was collected during the

Table 1. Amphibian and reptile species reported for Mount Lely, Suriname. Data are included from three main sources: the Rapid Assessment Program (“RAP 2007”), the current survey conducted in June 2016 (“Current 2016”), and all others from literature surveys and online sources (“Other”). The latter include literature references, when available, and asterisks indicate the original collector(s), when known. For species only reported from one of these three sources, the table cells are color coded (yellow, green, or blue), which emphasizes the inherently “incomplete” nature of any individual survey. The last column (“Seq”) indicates whether sequences are available.

Higher taxa	Species	RAP 2007	Current 2016	Other	Original collector/literature citation	Seq
Amphibia: Anura						
Aromobatidae	<i>Anomaloglossus stepheni</i>		X			
Aromobatidae	<i>Allobates femoralis</i>		X	X	Hoogmoed 1975*	X
Aromobatidae	<i>Allobates granti</i>		X	X	Noonan and Gaucher 2005	X
Bufonidae	<i>Rhinella marina</i>	X	X	X	Hoogmoed 1975*	X
Bufonidae	<i>Rhinella martyi</i>		X	X	Fouquet et al. 2007	X
Bufonidae	<i>Rhinella margaritifera</i> species complex	X				
Bufonidae	<i>Rhaebo guttatus</i>			X	Hoogmoed 1975*	
Bufonidae	<i>Atelopus hoogmoedi</i>			X	Ouboter and Jairam 2012	
Dendrobatidae	<i>Ameerega trivitata</i>	X	X			

Table 1 (continued). Amphibian and reptile species reported for Mount Lely, Suriname. Data are included from three main sources: the Rapid Assessment Program (“RAP 2007”), the current survey conducted in June 2016 (“Current 2016”), and all others from literature surveys and online sources (“Other”). The latter include literature references, when available, and asterisks indicate the original collector(s), when known. For species only reported from one of these three sources, the table cells are color coded (yellow, green, or blue), which emphasizes the inherently “incomplete” nature of any individual survey. The last column (“Seq”) indicates whether sequences are available.

Higher taxa	Species	RAP 2007	Current 2016	Other	Original collector/literature citation	Seq
Amphibia: Anura						
Hylidae	<i>Boana boans</i>		X	X	Hoogmoed 1975*	X
Hylidae	<i>Boana xerophylla</i>		X			X
Hylidae	<i>Dendropsophus leucophyllatus</i>		X	X	Hoogmoed 1975*	
Hylidae	<i>Dendropsophus minutus</i>	X	X	X	Hoogmoed 1975*	
Hylidae	<i>Dendropsophus melanargyreus</i>			X	Hoogmoed and Avila Pires 1991	
Hylidae	<i>Dendropsophus gaucheri</i>			X	Fouquet et al. 2011	
Hylidae	<i>Osteocephalus oophagus</i>		X			
Hylidae	<i>Osteocephalus taurinus</i>	X		X	Hoogmoed 1975*	
Hylidae	<i>Osteocephalus leprieuri</i>			X	Ouboter and Jairam 2012	
Hylidae	<i>Osteocephalus helenae</i>			X	Hoogmoed and Polder 1975*	
Phyllomedusidae	<i>Pithecopus hypochondrialis</i>		X	X	Myers 1975	
Phyllomedusidae	<i>Callimedusa tomopterna</i>		X	X	Hoogmoed 1975*	
Phyllomedusidae	<i>Phyllomedusa bicolor</i>		X	X	Hoogmoed 1975*	X
Phyllomedusidae	<i>Phyllomedusa vaillantii</i>			X	Hoogmoed 1975*	
Hylidae	<i>Scinax ruber</i>		X			
Hylidae	<i>Scinax proboscideus</i>			X	Hoogmoed 1975*	
Hylidae	<i>Scinax boesemani</i>			X	Hoogmoed 1975*	
Hylidae	<i>Trachycephalus typhonius</i>		X			
Leptodactylidae	<i>Adenomera</i> sp.	X				
Leptodactylidae	<i>Adenomera andreae</i>	X	X			X
Leptodactylidae	<i>Adenomera heyeri</i>		X	X	Hoogmoed and Polder 1975*; Fouquet et al. 2011	X
Leptodactylidae	<i>Adenomera hylaedactylus</i>		X			
Leptodactylidae	<i>Leptodactylus knudseni</i>	X	X			
Leptodactylidae	<i>Leptodactylus leptodactyloides</i>	X				
Leptodactylidae	<i>Leptodactylus longirostris</i>	X	X			X
Leptodactylidae	<i>Leptodactylus mystaceus</i>	X	X	X	Hoogmoed and Polder 1975*	
Leptodactylidae	<i>Leptodactylus pentadactylus</i>	X	X	X	Hoogmoed 1975*	
Leptodactylidae	<i>Leptodactylus stenodema</i>			X	Hoogmoed and Myers 1975*	
Leptodactylidae	<i>Leptodactylus guianensis</i>		X	X	Hoogmoed 1975*	X
Leptodactylidae	<i>Leptodactylus rhodomystax</i>		X	X	Hoogmoed and Polder 1975*	X
Leptodactylidae	<i>Leptodactylus petersii</i>			X	Ouboter and Jairam 2012	
Strabomantidae	<i>Pristimantis</i> cf. <i>inguinalis</i>	X				
Strabomantidae	<i>Pristimantis inguinalis</i>			X	Ouboter and Jairam 2012	
Strabomantidae	<i>Pristimantis marmoratus</i>			X	Myers 1975	
Strabomantidae	<i>Pristimantis</i> sp1	X				
Strabomantidae	<i>Pristimantis</i> sp2	X				
Strabomantidae	<i>Pristimantis</i> sp3	X				
Strabomantidae	<i>Pristimantis</i> sp4	X	X			X
Strabomantidae	<i>Pristimantis zeuctotylus</i>	X	X	X	Hoogmoed 1974*	X

Herpetofauna of the Lely Mountains, Suriname

Table 1 (continued). Amphibian and reptile species reported for Mount Lely, Suriname. Data are included from three main sources: the Rapid Assessment Program (“RAP 2007”), the current survey conducted in June 2016 (“Current 2016”), and all others from literature surveys and online sources (“Other”). The latter include literature references, when available, and asterisks indicate the original collector(s), when known. For species only reported from one of these three sources, the table cells are color coded (yellow, green, or blue), which emphasizes the inherently “incomplete” nature of any individual survey. The last column (“Seq”) indicates whether sequences are available.

Higher taxa	Species	RAP 2007	Current 2016	Other	Original collector/literature citation	Seq
Amphibia: Anura						
Strabomantidae	<i>Pristimantis chiastonotus</i>			X	Hoogmoed 1974*	
Strabomantidae	<i>Pristimantis gutturalis</i>			X	Ouboter and Jairam 2012	
Microhylidae	<i>Chiasmocleis shudikarensis</i>	X	X			
Pipidae	<i>Pipa aspera</i>			X	G.F. Mees 1979*	
Amphibia: Gymnophiona						
Caecilidae	<i>Microcaecilia grandis</i>			X	Wilkinson et al. 2009	
Total amphibians	49 species	19	28	33		
Reptilia: Sauria						
Phyllodactylidae	<i>Thecadactylus rapicauda</i>		X			
Gekkonidae	<i>Lepidodactylus lugubris</i>		X			
Gekkonidae	<i>Hemidactylus mabouia</i>		X			
Sphaerodactylidae	<i>Gonatodes annularis</i>	X				
Sphaerodactylidae	<i>Gonatodes humeralis</i>	X	X	X	Hoogmoed 1974*	
Gymnophthalmidae	<i>Loxopholis guianense</i>	X	X	X	Hoogmoed 1974*	
Gymnophthalmidae	<i>Neusticurus bicarinatus</i>		X	X	Hoogmoed and Polder 1975*	
Gymnophthalmidae	<i>Neusticurus rudis</i>	X		X	Hoogmoed 1974*	
Gymnophthalmidae	<i>Arthrosaura reticulata</i>			X	Hoogmoed and Avila-Pires 1992	
Gymnophthalmidae	<i>Arthrosaura kocki</i>		X	X	Hoogmoed and Avila-Pires 1992	
Gymnophthalmidae	<i>Arthrosaura versteegi</i>			X	Hoogmoed and Avila-Pires 1992	
Dactyloidae	<i>Anolis chrysolepis</i>	X		X	Hoogmoed 1975*	
Dactyloidae	<i>Anolis punctatus</i>			X	Hoogmoed 1975*	
Polychrotidae	<i>Polychrus marmoratus</i>		X			
Scincidae	<i>Copeoglossum nigropunctatum</i>	X		X	Hoogmoed 1975*	
Gymnophthalmidae	<i>Iphisa elegans</i>			X	Hoogmoed 1975*	
Gymnophthalmidae	<i>Tretioscincus agilis</i>			X	Hoogmoed 1975*	
Spaerodactylidae	<i>Chatogekko amazonicus</i>			X	Hoogmoed 1975*	
Teiidae	<i>Ameiva ameiva</i>	X	X	X	Hoogmoed 1975*	
Teiidae	<i>Kentropix calcarata</i>	X				
Teiidae	<i>Tupinambis teguixin</i>	X	X			
Tropiduridae	<i>Plica plica</i>	X		X	G.F. Mees 1979*	
Tropiduridae	<i>Plica umbra</i>		X	X	Hoogmoed 1975*	
Reptilia: Crocodylia						
Alligatoridae	<i>Paleosuchus trigonatus</i>	X	X			
Alligatoridae	<i>Caiman crocodilus</i>			X	Hoogmoed 1975*	
Reptilia: Serpentes						
Leptotyphlopidae	<i>Siagonodon cupinensis</i>			X	Hoogmoed 1977	
Leptotyphlopidae	<i>Epictia tenella</i>		X			

Table 1 (continued). Amphibian and reptile species reported for Mount Lely, Suriname. Data are included from three main sources: the Rapid Assessment Program (“RAP 2007”), the current survey conducted in June 2016 (“Current 2016”), and all others from literature surveys and online sources (“Other”). The latter include literature references, when available, and asterisks indicate the original collector(s), when known. For species only reported from one of these three sources, the table cells are color coded (yellow, green, or blue), which emphasizes the inherently “incomplete” nature of any individual survey. The last column (“Seq”) indicates whether sequences are available.

Higher taxa	Species	RAP 2007	Current 2016	Other	Original collector/literature citation	Seq
Reptilia: Serpentes						
Anilliidae	<i>Anilius scytale</i>			X	no data on collector	
Boidae	<i>Corallus caninus</i>			X	G.F. Mees 1979*	
Colubridae	<i>Atractus badius</i>			X	Hoogmoed 1975*	
Colubridae	<i>Dipsas catesbyi</i>	X				
Colubridae	<i>Dipsas indica</i>	X				
Colubridae	<i>Imantodes</i> sp.	X				
Colubridae	<i>Mastigodryas boddaerti</i>		X			
Colubridae	<i>Chironius carinatus</i>		X			
Colubridae	<i>Chironius fuscus</i>			X	G.F. Mees 1979*	
Colubridae	<i>Oxyrhopus formosus</i>	X				
Viperidae	<i>Bothrops atrox</i>	X	X			
Viperidae	<i>Bothrops brazili</i>			X	Lindeman 1975*	
Viperidae	<i>Bothrops bilineatus</i>	X				
Reptilia: Testudines						
Chelidae	<i>Platemys platycephala</i>	X	X	X	Hoogmoed 1975*	
Kinosternidae	<i>Kinosternon scorpioides</i>		X	X	Hoogmoed 1975*	
Total reptiles	42 species	18	18	25		

June 2016 survey. All specimens observed were found around the houses on water tanks and rain gauges at the airstrip. Although this species was observed and possibly collected by the members of the RAP, the specimens were misidentified as an undescribed *Pristimantis* species. For example, a misidentified specimen is depicted on the cover of the RAP report (Alonso and Mol 2007).

- *Osteocephalus oophagus* was heard calling while surveyors were walking at night in a patch of undisturbed forest south of the airfield in the Lely Mountains.
- *Scinax ruber* specimens were collected for the first time for the Lely Mountains. Specimens were observed on the large water holding tanks found around the houses on the airstrip.
- A specimen of *Chironius carinatus* was collected near one of the buildings at the airstrip on the tap of one of the water tanks.
- A specimen of *Trachycephalus typhonius* was collected during a night survey while surveyors were walking on a Suralco road. The collected individual was sitting on a leaf approximately 1.5 m above the

ground in a habitat of undisturbed forest.

- *Pristimantis* sp. 4 is noted for the first time for the Lely Mountains.
- *Thecadactylus rapicauda* was found on some of the buildings at the airstrip, apparently having moved to these locations from the surrounding forests.
- *Lepidodactylus lugubris* is an introduced SE Asian gecko (Hoogmoed and Avila-Pires 2015), and was most probably introduced into the Lely Mountains by the frequent flights which supply the small-scale gold miners and the airstrip personnel.
- *Hemidactylus mabouia* represents the first record for yet another introduced species in the Lely Mountains, and was frequently observed on the building where the surveyors stayed.
- *Polychrus marmoratus* was collected for the first time for this location, near the edge of the airstrip.
- Two specimens of *Epictia tenella* were collected in the morning around 8 AM, while the surveyors were walking around the edges of the airstrip. Both speci-

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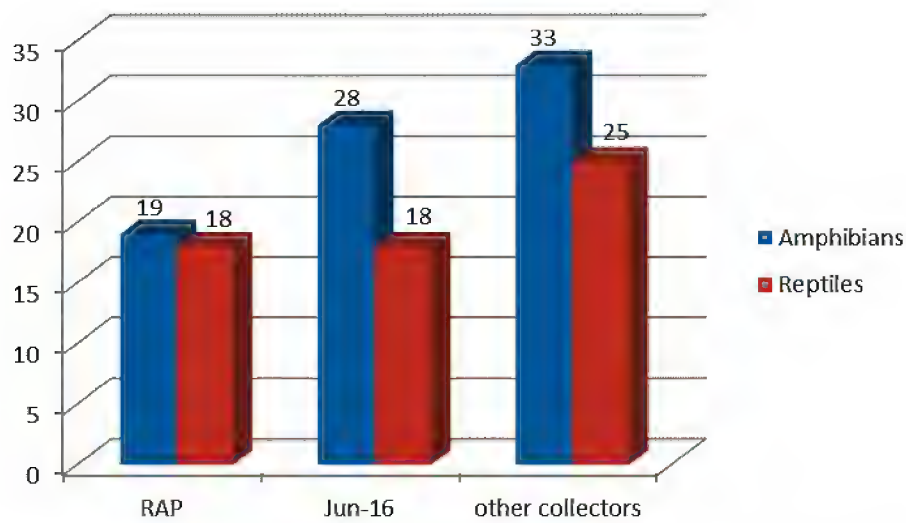


Fig. 2. Total number of species per group collected during each of the major surveys in the Lely Mountains, Suriname. “RAP” is the Rapid Assessment Program survey (Alonso and Mol 2007); “Jun-16” is the current survey; “Other collectors” includes all other available data.

mens were near the grassy edges of the airstrip and were active when observed.

- *Mastigodryas boddaerti* was observed whilst lifting a corrugated plate left behind in one of the abandoned small-scale miner camps.
- The pristine forests around the airstrip contained relatively large, water-filled depressions which were attractive to species such as *Chiasmocleis shudikarensis* and *Platemys platycephala*.
- Roads on the plateau with large water-filled potholes were an ideal habitat for *Phyllomedusa bicolor*, *Calimeda tomopterna*, *Pithecopus hypochondrialis*, and other Hylidae.



Fig. 3. (A) *Anomaloglossus stepheni*; (B) *Allobates femoralis*; (C) *Allobates granti*; (D) *Rhinella martyi*; (E) *Boana xerophylla*; and (F) *Scinax ruber*.



Fig. 4. (A) *Trachycephalus typhonius*; (B) *Leptodactylus knudseni* (juv.); (C) *Leptodactylus pentadactylus* (juv.); (D) *Leptodactylus mystaceus*; (E) *Leptodactylus rhodomystax* (juv.); and (F) *Ameerega trivittata*.

Discussion

Some problems were encountered with two of the species collected during the RAP, namely *Anomaloglossus beebei* and *Anomaloglossus degranvillei*. The authors of the RAP survey confused *A. beebei* with *Allobates granti*, which occurs in the Lely Mountains whilst the former is only found at Kaieteur National Park, Guyana (Kok et al. 2006). The *Anomaloglossus degranvillei* reportedly collected during the RAP might have been confused with *A. stepheni* or *A. surinamensis*. A study by Fouquet et al. (2018) has shown that *A. degranvillei* is restricted to a small area in French Guiana and also proved that *A. surinamensis* probably occurs in the Lely Mountains. Specimens of *A. stepheni* were collected during the 2016 survey. *Anomaloglossus surinamensis* was first described

by Ouboter and Jairam (2012) who had previously also identified specimens from the nearby Nassau Mountains as *A. degranvillei* (Ouboter et al. 2007).

Some of the *Pristimantis* species documented during the RAP still remain to be identified, so chances are that their eventual identification might change the number of amphibian species for the Lely Mountains. Since the 2016 survey was held in a period with very little rainfall, very few frogs were calling. Therefore, we believe that additional surveys during the rainy season would be interesting and would definitely increase the number of known amphibians for this location.

The increasing human presence in the Lely Mountains might have resulted in an extension of the distribution ranges for *Boana sclerophylla*, *Scinax ruber*, *Trachycephalus typhonius*, *Lepidodactylus lugubris*, and



Fig. 5. (A) *Leptodactylus longirostris*; (B) *Boana boans*; (C) *Callimedusa tomopterna*; (D) *Phyllomedusa bicolor*; and (E) *Pithecopus hypochondrialis*. Photos by D. Baeta.

of species present and to save this Mountain from the complete destruction of its habitats.

Conclusions

The compiled results presented herein show that the amphibian and reptile communities on the Lely Mountains are much more diverse than previously reported. Though five separate surveys have been conducted at this locality, chances are that the known species diversity will continue to increase as different areas on the plateau are surveyed. The presence of illegal small-scale gold miners on the plateau has resulted in the rapid conversion of forested areas into unsuitable habitats which may translate into a loss of species diversity.

Acknowledgements.—I would like to thank the Nature Conservation Department that kindly provided the permits which allowed the fieldwork in the Lely Mountains. D. Baéta and T. Gazoni were valuable companions in the field, and contributed significantly to the number of species documented during the survey in June 2016. A first draft of this article was reviewed by A. Fouquet whose comments greatly improved the manuscript.

Hemidactylus mabouia; species that were not recorded during the earlier surveys. Although most of the days spent on the Lely Mountains were quite dry not many reptile species were collected. Snakes, for example, were the least represented group, and just four specimens belonging to three species were found. Small-scale gold miners were very active in the Lely Mountains, a factor which might have contributed to the disturbance in the forest and the only creek that was found and sampled. The number of species added to the list published during the RAP and the prospects for organizing another survey to this location validate the value of this checklist. We would strongly recommend that the Lely Mountains be spared from further destruction by illegal mining activities, and additional surveys involving other groups should be conducted to establish a satisfactory list



Fig. 6. (A) *Adenomera heyeri*; (B) *Dendropsophus leucophyllatus*; (C) *Chiasmocleis shudikarensis*; and (D) *Pristimantis* sp. 4.

Literature Cited

- Alonso LE, Mol JH (Editors). 2007. *A Rapid Biological Assessment of the Lely and Nassau Plateaus, Suriname (with Additional Information on the Brownsberg Plateau)*. RAP Bulletin of Biological Assessment 43. Conservation International, Center for Applied Biodiversity Science (CABS), Arlington, Virginia, USA. 276 p.
- Avila-Pires TCS. 1995. Lizards of Brazilian Amazonia (Reptilia: Squamata). *Zoologische Verhandelingen* 299: 1–706.
- Bauer AM, Jackman TR, Greenbaum E, Papenfuss TJ. 2007. First record of *Lepidodactylus lugubris* in Suriname. *Applied Herpetology* 4(1): 84.
- Fouquet A, Gaucher P, Blanc M, Velez-Rodriguez CM. 2007. Description of two new species of *Rhinella* (Anura: Bufonidae) from the lowlands of the Guiana Shield. *Zootaxa* 1663: 17–32.
- Fouquet A, Vacher J-P, Kadosoe V, Ouboter P, Jairam R. 2015. Checklist of the amphibians of the Sipaliwini area, Suriname. *Herpetology Notes* 8: 63–68.
- Fouquet A, Noonan BP, Blanc M, Orrico VGD. 2011. Phylogenetic position of *Dendropsophus gaucheri* (Lescure and Marty 2000) highlights the need for an in-depth investigation of the phylogenetic relationships of *Dendropsophus* (Anura: Hylidae). *Zootaxa* 3035: 59–67.
- Fouquet A, Orrico VGD, Ernst R, Blanc M, Martinez Q, Vacher JP, Rodriques MT, Ouboter P, Jairam R, Ron S. 2015. A new *Dendropsophus* Fitzinger, 1843 (Anura: Hylidae) of the *parviceps* group from the lowlands of the Guiana Shield. *Zootaxa* 4052(1): 39–64.
- Hoogmoed MS. 1973. *Notes on the Herpetofauna of Surinam IV: The Lizards and Amphisbaenians of Surinam*. Biogeographica 4. W. Junk Publishers, The Hague, Netherlands. 419 p.
- Hoogmoed MS, Avila-Pires TCS. 2015. *Lepidodactylus lugubris* (Duméril and Bibron 1836) (Reptilia: Gekkonidae), an introduced lizard new for Brazil, with remarks on and correction of its distribution in the New World. *Zootaxa* 4000(1): 90–110.
- Jairam R, Jairam-Doerga S. 2016. Range extension and some morphological characteristics of *Ptychoglossus brevifrontalis* Boulenger, 1912 (Squamata: Alopoglossidae) in Suriname. *Amphibian & Reptile Conservation* 10(2) [General Section]: 30–33 (e127).
- Jairam R, Jairam-Doerga S. 2015. First record of *Amapasaurus tetradactylus* Cunha, 1970 (Squamata: Gymnophthalmidae) in Suriname. *Check List* 11: 1–4.
- Jairam R, d'Orgeix CA, d'Orgeix CH, Harris A. 2016. Range extension and distribution of the invasive Moreau's Tropical House Gecko, *Hemidactylus mabouia* (Moreau de Jonnès, 1818) (Squamata: Gekkonidae), in Suriname. *Check List* 12(5): 1–5.
- Kok PJR, Sambhu H, Roopsind I, Lenglet GL, Bourne GR. 2006. A new species of *Colostethus* (Anura: Dendrobatidae) with maternal care from Kaieteur



Fig. 7. (A) *Arthrosaura kocki*; (B) *Loxopholis guianense*; (C) *Neusticurus bicarinatus*; (D) *Polychrus marmoratus*; and (E) *Plica umbra*.

National Park, Guyana. *Zootaxa* 1238: 35–61.

Mol JH, Wan Tong You K, Vrede I, Flynn A, Ouboter P, van der Lugt F. 2007. Fishes of Lely and Nassau Mountains, Suriname. Pp. 107–118 In: *A Rapid Assessment of the Lely and Nassau Plateaus, Suriname (with Additional Information on the Brownsberg Plateau)*. RAP Bulletin of Biological Assessment 43. Editors, Alonso LE, Mol JH. Conservation International, Center for Applied Biodiversity Science (CABS), Arlington, Virginia, USA. 276 p.

Nielsen S, Jairam R, Ouboter P, Noonan B. 2013. A

herpetofaunal survey of the Grensgebergte and Kasikasima regions, Suriname. Pp. 131–144 In: *A Rapid Biological Assessment of the Upper Palumeu Watershed (Grensgebergte and Kasikasima) of Southeastern Suriname*. RAP Bulletin of Biological Assessment 67. Editors, Alonso LE, Larsen TH. Conservation International, Arlington, Virginia, USA. 176 p.

Noonan BP, Gaucher P. 2005. Phylogeography and demography of Guianan harlequin toads (*Atelopus*): diversification within a refuge. *Molecular Ecology*



Fig. 8. (A) *Chironius fuscus*; and (B) *Epictia tenella*.

14(10): 3,017–3,031.

Ouboter P. 2017. Amphibians and reptiles. Pp. 256–303 In: *Natural History and Ecology of Suriname*. Editor, de Dijn B. LM Publishers, Volendam, Netherlands. 480 p.

Ouboter PE, Jairam R, Kasanpawiro C. 2011. A rapid assessment of the amphibians and reptiles of the Kwamalasamutu region (Kutari/lower Sipaliwini Rivers), Suriname. Pp. 124–130 In: *A Rapid Biological Assessment of the Kwamalasamutu Region, Southwestern Suriname*. RAP Bulletin of Biological Assessment 63. Editors, O'Shea BJ, Alonso LE, Larsen TH. Conservation International, Arlington, Virginia, USA. 156 p.

Ouboter PE, Jairam R. 2012. *Amphibians of Suriname*. Brill Academic, Leiden, Netherlands. 376 p.

Ouboter PE, Jairam R, Wan Tong You K. 2007. Additional records of amphibians from Nassau Mountains, Suriname. Pp. 126–129 In: *A Rapid Assessment of the Lely and Nassau Plateaus, Suriname (with Additional Information on the Brownsberg Plateau)*. RAP Bulletin of Biological Assessment 43. Editors, Alonso LE, Mol JH. Conservation International, Center for

Applied Biodiversity Science (CABS), Arlington, Virginia, USA. 276 p.

Powell R, Crombie RI, Boos HE. 1998. *Hemidactylus mabouia*. *Catalogue of American Amphibians and Reptiles* 674: 1–11.

Vacher JP, Kok PJ, Rodrigues MT, Lima JD, Lorenzini A, Martinez Q, Fallet M, Courtois EA, Blanc M, Gaucher P, et al. 2017. Cryptic diversity in Amazonian frogs: integrative taxonomy of the genus *Anomaloglossus* (Amphibia: Anura: Aromobatidae) reveals a unique case of diversification within the Guiana Shield. *Molecular Phylogenetics and Evolution* 112: 158–173.

Watling JI, Ngadino LF. 2007. A preliminary survey of amphibians and reptiles on Nassau and Lely Mountains, eastern Suriname. Pp. 119–125 In: *A Rapid Biological Assessment of the Lely and Nassau Plateaus, Suriname (with Additional Information on the Brownsberg Plateau)*. RAP Bulletin of Biological Assessment 43. Editors, Alonso LE, Mol JH. Conservation International, Center for Applied Biodiversity Science (CABS), Arlington, Virginia, USA. 276 p.



Fig. 9. Overview of the airstrip in the Lely Mountains with parts of the forest visible where the June 2016 surveys were conducted. Photo by T. Gazoni.



Rawien Jairam is an associate researcher working at the National Zoological Collection of Suriname. Rawien has an M.Sc. in conservation biology and has been interested in the herpetofauna of Suriname for many years. In addition to herpetology in general, he is specifically interested in taxonomy, species descriptions, and distribution.



Book Review

Islands and Snakes: Isolation and Adaptive Evolution

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Keywords. Behavior, biogeography, ecology, reproduction, reptiles, Serpentes, Squamata

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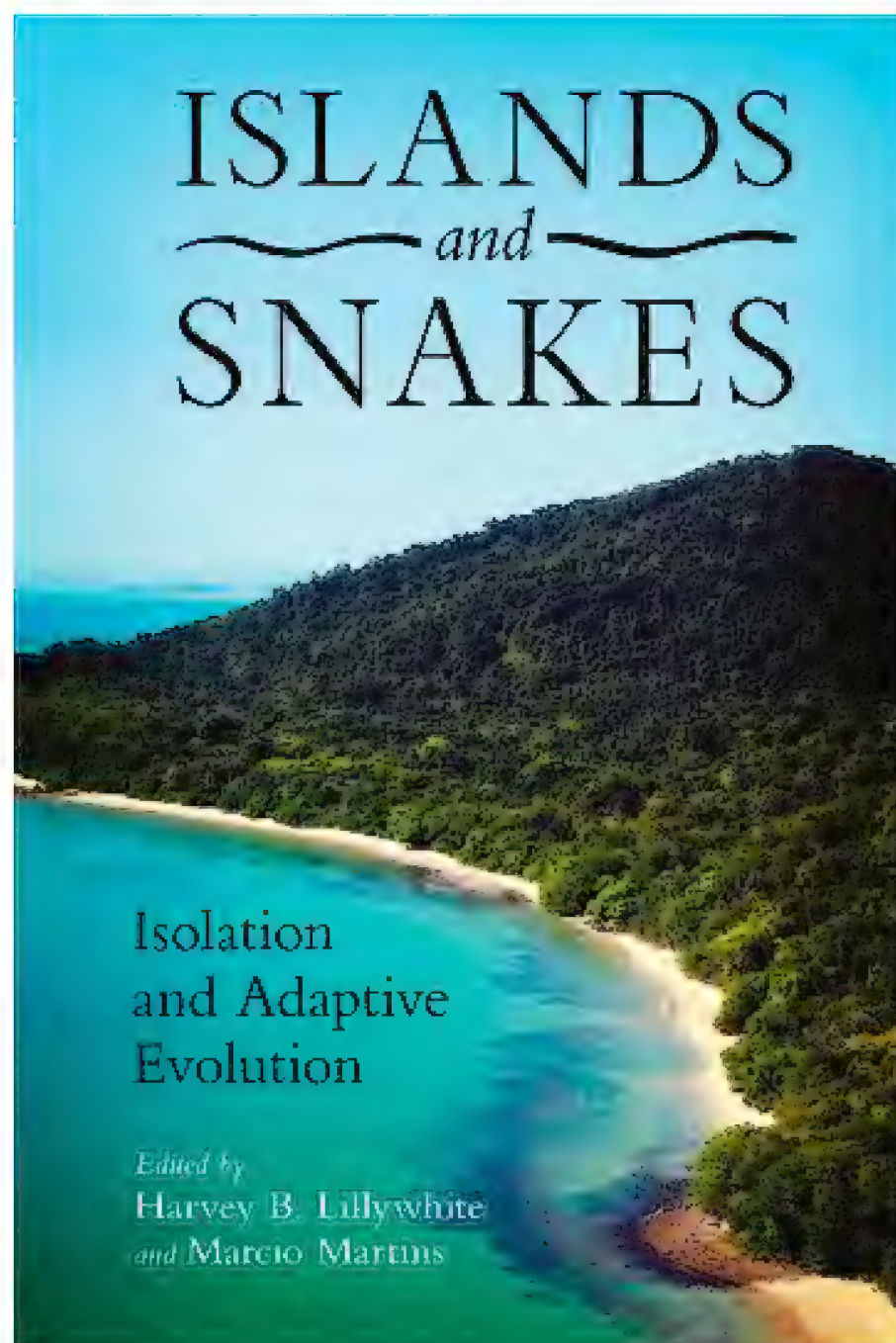
Snakes on islands, what could make a herpetologist happier? *Islands and Snakes*, edited by Harvey B. Lillywhite and Marcio Martins, is a fun and important book, with something new and fascinating in every chapter: A tropical island with Sea Kraits coming ashore to drink fresh water and to lay their eggs; a sandy Florida beach, where at the back-beach vegetation, dozens of Cottonmouth Moccasins wait for falling baby birds or for the dropped fish that the parent birds had brought to their young. That and so much more is here in this exciting book!

Islands are fascinating, as each has its own ecology. Isolated and oceanic islands have depauperate faunas due to distance and dispersal. Continental islands have fairly normal ecosystems, though some species may be missing and others becoming dominant. The authors of the chapters in this book show how interesting snakes on islands have become.

The introductory chapter by Marcio Martins and Harvey Lillywhite discusses the geology, geography, and evolution of islands and their snake fauna, followed by Harold Heatwole's chapter on the biogeography of Sea Kraits, and then the chapter by Xavier Bonnet and François Brischoux on Sea Krait behavior, distribution, and abundance. Fun facts: Sea Kraits can pick up one-fifth of their oxygen through their skin, they eat mostly eels, they must go ashore on islands to lay their eggs, they form big mating balls, and many have predatory ticks.

In Chapter 4, by Ming-Chung Tu and Harvey Lillywhite, we learn more about the diving responses of Sea Kraits and the fact that after a rain, Kraits can drink from the tiny layer of freshwater that has fallen on the ocean surface...and you will also learn more about the mating balls.

In Chapter 5, by Marcio Martins, Ricardo J. Sawaya, Selma Almeida-Santos, and Otavio A.V. Marqués, we learn about the ecology of the Lancehead, *Bothrops*



Title: *Islands and Snakes: Isolation and Adaptive Evolution*

Editors: Harvey B. Lillywhite and Marcio Martins

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insularis, on a Brazilian Island; followed by Chapter 6 by Fabien Aubret on the elapid Tiger Snake, *Notechis scutatus*, on one of the islands between Australia and Tasmania, which is the breeding site of several species of sea birds. With all this available food (shearwaters, petrels, gulls, cormorants, skinks, and mice), these snakes get to be as large as 1.5 m and become a hazard for the scientists that are studying the birds!

In Chapter 7 by Robert Henderson we learn about the Tree Boa, *Corallus grenadensis*, followed in Chapter 8 by a study of the ecology and variation in the Milos Viper, *Macrovipera schwizeri*, by Göran Nilson.

Chapter 9, by Harvey Lillywhite and Coleman Sheehy III, continues the important studies on Cottonmouth Moccasins, *Agkistrodon piscivorus*, including their eating baby birds and dropped fish on an island off the coast of Florida, USA. Richard B. King and Kristin M. Stanford bring us up to date in Chapter 11 on the decades-long studies on Water Snakes, *Nerodia sipedon insularum*, their ecology, and evolution on Lake Erie islands between

the USA and Canada, showing both positive and negative human impacts on the snakes.

On Catalina Island in the Gulf of California, there is a rattlesnake that has no rattle, *Crotalus catalinensis*, and Chapter 10 by Gustavo Arnaud and Marcio Martins covers this snake's behavior, ecology, and conservation; and suggests that a native Night Snake, *Hypsiglena catalinae*, might be a color mimic of this rattle-less rattlesnake. Chapter 12, by Akira Mori, H. Ota, and Koichi Hirate discusses the impact of snakes eating baby sea turtles that are on their way from the nest to the sea. Since there are "islands" of habitat (deserts, ponds, areas between lava flows or between rivers), D. Bruce Means and César Barrio-Amorós discuss in Chapter 13 the snakes on the South American Sky Islands—the Tepuis—with interesting results.

Bottom line: The book is well written by all the authors, the pictures are for the most part quite good, and the information is fun and exciting. BUY IT!



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Possible hybridization between East Pacific Green *Chelonia mydas* and Olive Ridley *Lepidochelys olivacea* sea turtles in northwest Mexico

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Abstract.—Photographic records of sea turtle neonates and embryos which show characteristics of both East Pacific Green Sea Turtles (*Chelonia mydas*) and Olive Ridley Sea Turtles (*Lepidochelys olivacea*) are presented. These turtles were produced from nests laid by Olive Ridley females in the states of Nayarit, Jalisco, and Baja California Sur. Their discovery further suggests the occurrence of hybridization between these two species, and potential implications for conservation are discussed.

Keywords. Black sea turtle, Gulf of California, morphology, conservation, hybrid, Testudines

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Introduction

Sea turtles are widely distributed throughout tropical and subtropical oceans. They nest on sandy beaches throughout their range, and while different species can be found sharing habitats worldwide each has adapted to take advantage of different ecological niches (Tomas et al. 2001; Ballorain et al. 2010; Jones et al. 2012). Despite this, where species of the same lineage coincide in time and space mating and inter-specific hybridization sometimes occurs naturally, a process which influences 25% of plant and 10% of animal species (Arnold 1997). When hybridization involves threatened or endangered species it is considered to be of conservation concern (Allendorf et al. 2001), and there is a need to determine whether these events are a result of anthropogenic factors before implementing management strategies (Genovart 2009). Of the seven extant species of sea turtles, hybridization has been reported between Loggerheads (*Caretta caretta*) and Kemp's (*Lepidochelys kempii*) [Barber et al. 2003]; Loggerheads and Hawksbills (*Eretmochelys imbricata*) [Lara-Ruiz et al. 2006]; Greens (*Chelonia mydas*) and Loggerheads (James et al. 2004); Greens and Hawksbills

(Seminoff et al. 2003; Kelez et al. 2016); and Hawksbills and Olive Ridleys (*L. olivacea*) [Lara-Ruiz et al. 2006]. Thus, the Carettini and Chelonini tribes to which they belong are thought to be one of the oldest vertebrate lineages that is known to hybridize in nature (Karl et al. 1995), sharing a common ancestor more than 50 million yr ago (Bowen et al. 1993; Dutton et al. 1996).

Along the Pacific coast of Mexico, nesting occurs for four species of sea turtles: Leatherback, Hawksbill, Green, and Olive Ridley, with the latter being by far the most abundant. East Pacific Green Turtles are the second most abundant turtle to nest on Mexico's Pacific beaches, and they often nest alongside Olive Ridleys. These two species have overlapping breeding seasons with Olive Ridleys nesting from May to December (Garcia et al. 2003) and Greens from September to January (Alvarado-Díaz et al. 2003). Currently both species exhibit incipient recovery and nearly year-round nesting as a result of the population increases following decades of conservation activities. Extensive hunting and egg collection, which reached industrial levels in the 1970s and 1980s, led to drastic population declines (Chassin-Noria et al. 2004; Rodríguez-Zarate et al. 2013). These declines

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Fig. 1. Northwest Mexico. Circles denote nesting beaches where suspected hybrid hatchlings have been observed.

were reversed with a total ban on sea turtle use starting in 1990 (Marquez et al. 1998) and the proliferation of conservation programs. Improvements in the conditions of these species have resulted in both being moved from the Endangered to Vulnerable classifications on the IUCN Red List, although both species remain listed in Appendix I of CITES (2007).

Here, the possible hybridization of East Pacific Green Sea Turtles and Olive Ridley Turtles is reported in northwest Mexico based on hatchling characteristics.

Materials and Methods

During 2010–2012, hatchlings and embryos from 12 nests laid by different Olive Ridley females were observed with characteristics (see Table 1) typically associated with East Pacific Green Turtles. Conservation biologists collaborating with two NGOs in north-west Mexico, Red Tortuguera A.C. and Grupo Tortuguero de las Californias A.C., were requested to report embryos and neonates that were atypically pigmented, or those that presented scute or morphological patterns associated

with a species different than that of the female which had nested. Following the subsequent reports, photographic records of the neonates were made prior to their release.

Results

All of the hatchling sea turtles that presented atypical characteristics hatched from nests verified to be laid by Olive Ridley females. Figures 2A and 2B show the contrasting morphology and coloration of the carapace and plastron for Olive Ridley and East Pacific Green Turtle hatchlings, respectively. Embryos (Fig. 3) and hatchlings (Figs. 4–5) were occasionally found presenting coloration typical of East Pacific green turtle hatchlings. Interestingly these hatchlings were often the only abnormal hatchlings from an otherwise typical Olive Ridley clutch, with their siblings presenting typical Olive Ridley coloration.

Olive Ridley hatchlings presenting a white border to the marginal scutes and white edges to the flippers were frequently reported (Fig. 6). We consider this to be a normal characteristic, and not a sign of hybridization, that

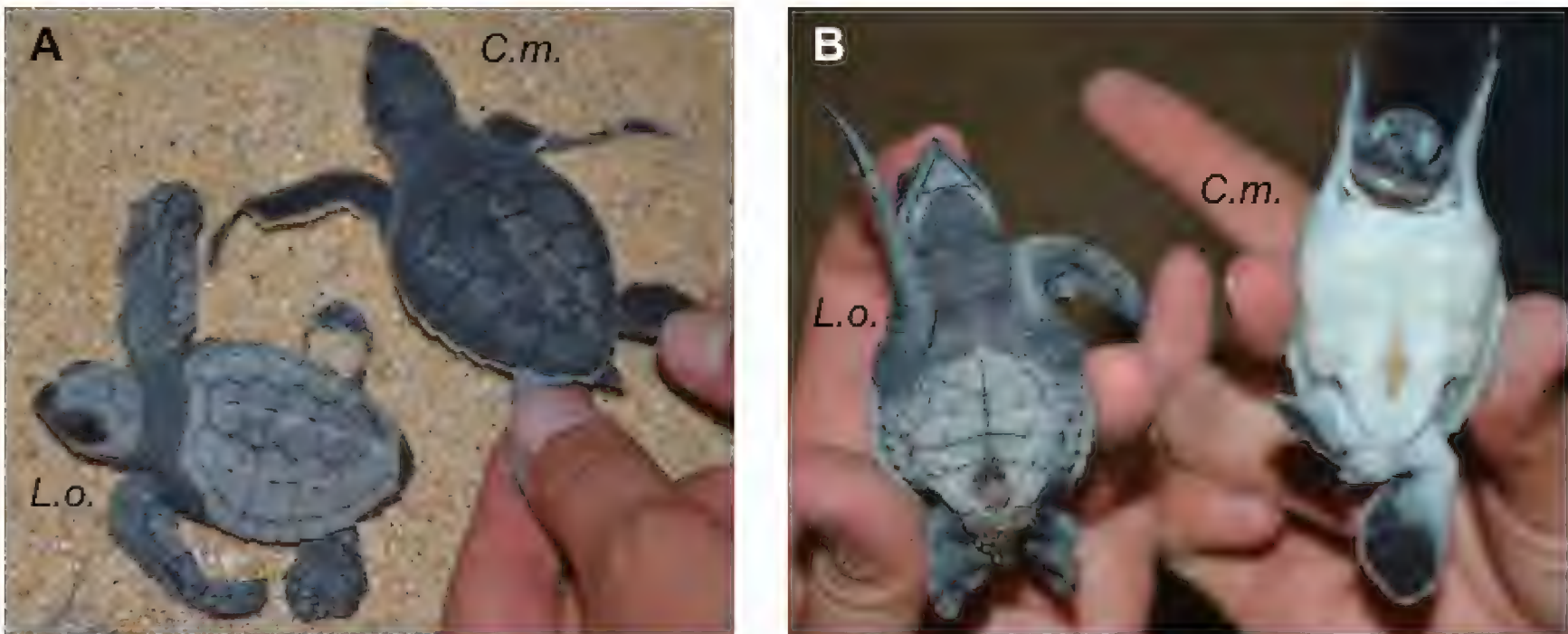


Fig. 2. Carapace (A) and plastron (B) of *Lepidochelys olivacea* (L.o.) and *Chelonia mydas* (C.m.) hatchlings.

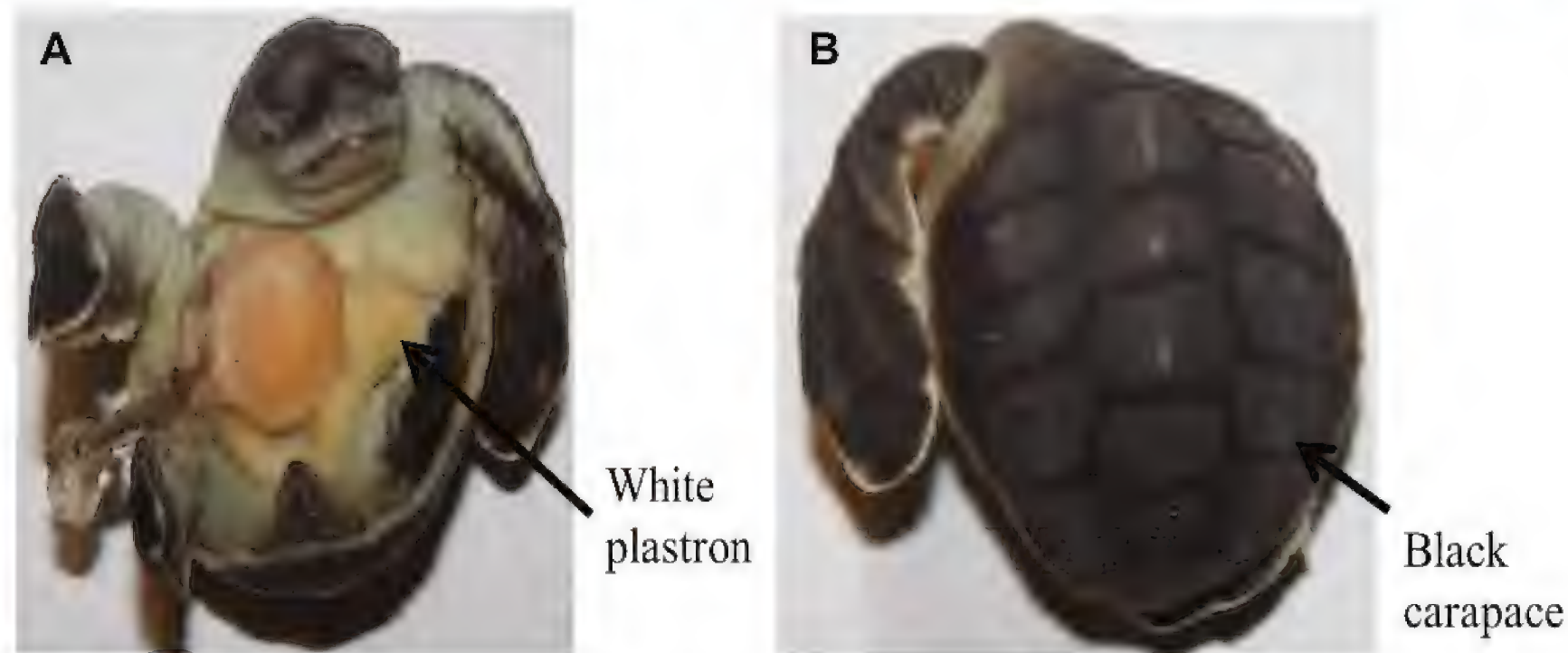


Fig. 3. Embryo from an Olive Ridley nest, clearly displaying East Pacific Green Turtle coloration on both (A) plastron and flippers, and (B) carapace. *Photos by C.E. Hart.*

Table 1. Morphological features of putative hybrid neonate turtles compared to those usually reported for *Lepidochelys olivacea* and *Chelonia mydas*.

Morphological feature	<i>L. olivacea</i>	<i>C. mydas</i>	Putative hybrids
Prefrontal scales	4	2	2 or 4
Post orbital scales	3	3	3
Marginal scutes	12	11	11–12
Supracaudal scutes	2	2	2
Intergular scute		Yes	
Postanal scute		No	
Nuchal scute	Yes	Yes	Yes
Lateral scutes	6–9*	4	4–8
Vertebral scutes	6–9	5	6–7
Inframarginal scutes		4	
Keels	Yes	No	Yes
Beak	Triangular	Rounded, large	Triangular or rounded
Anterior claws	2	1	1–2
Carapace color	Gray	Black	Dark gray or black
Plastron color	Gray	White	White

*occasionally five

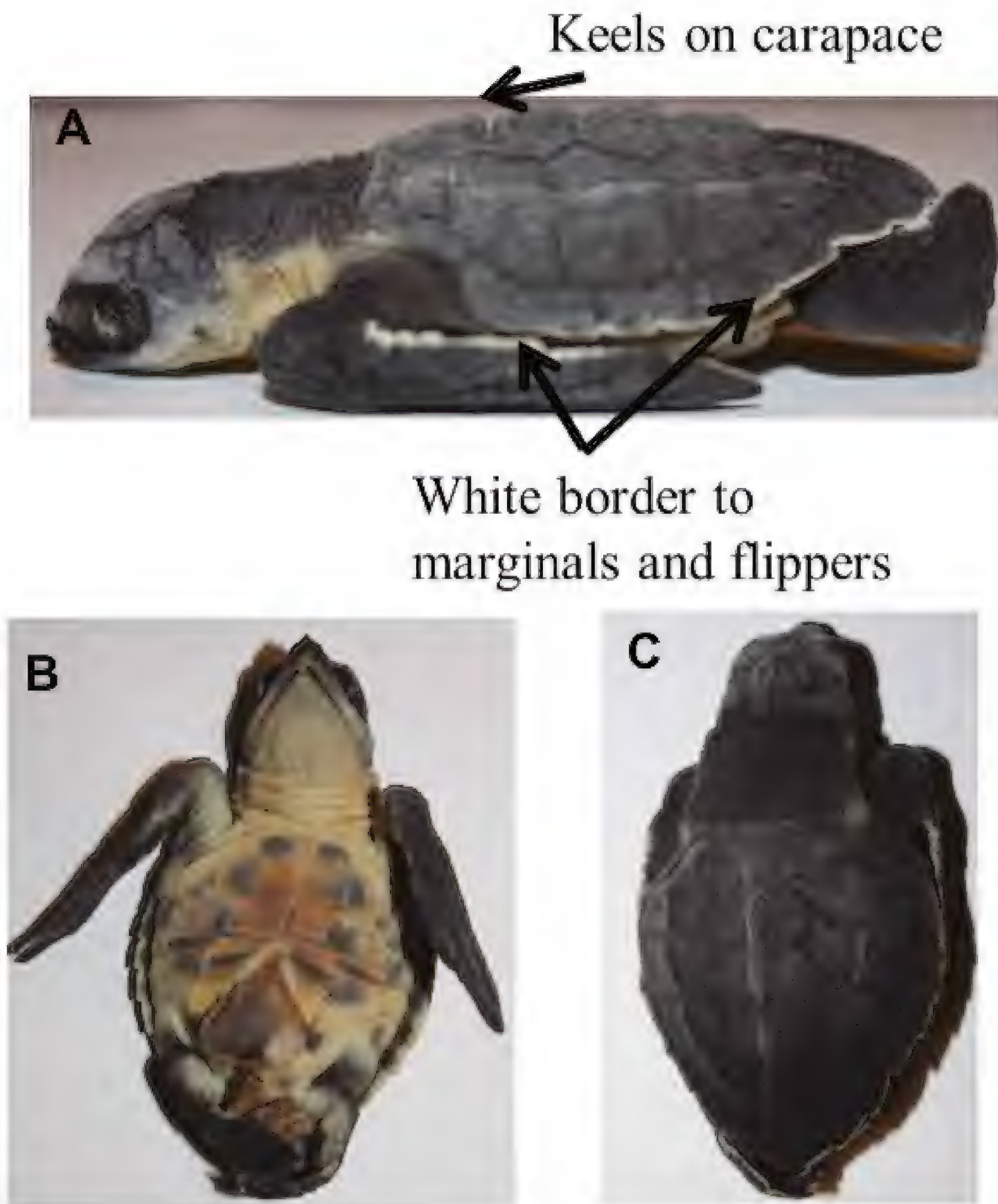


Fig. 4. Deceased hatchling presenting (A) white coloration to the carapace and flipper border, and (B) the white plastron characteristic of East Pacific Green Turtles, while presenting (C) a typical Olive Ridley carapace and head. *Photos by C.E. Hart.*



Fig. 5. Healthy neonate turtles presenting characteristics of both East Pacific Green and Olive Ridley Turtles photographed before release. *Photos by C.E. Hart (A) and F. Sanchez (B).*

is present in some but not all Olive Ridley neonates and is found within all participating hatcheries in southern Nayarit and north Jalisco. However, a white border on the marginal scutes and white edges to the flippers are characteristics associated with East Pacific Green

Turtle neonates, and they have not been described as pigmentation for Olive Ridley neonates. Genetic studies are needed to clarify whether these neonates are pure Olive Ridelies or the result of hybridization.

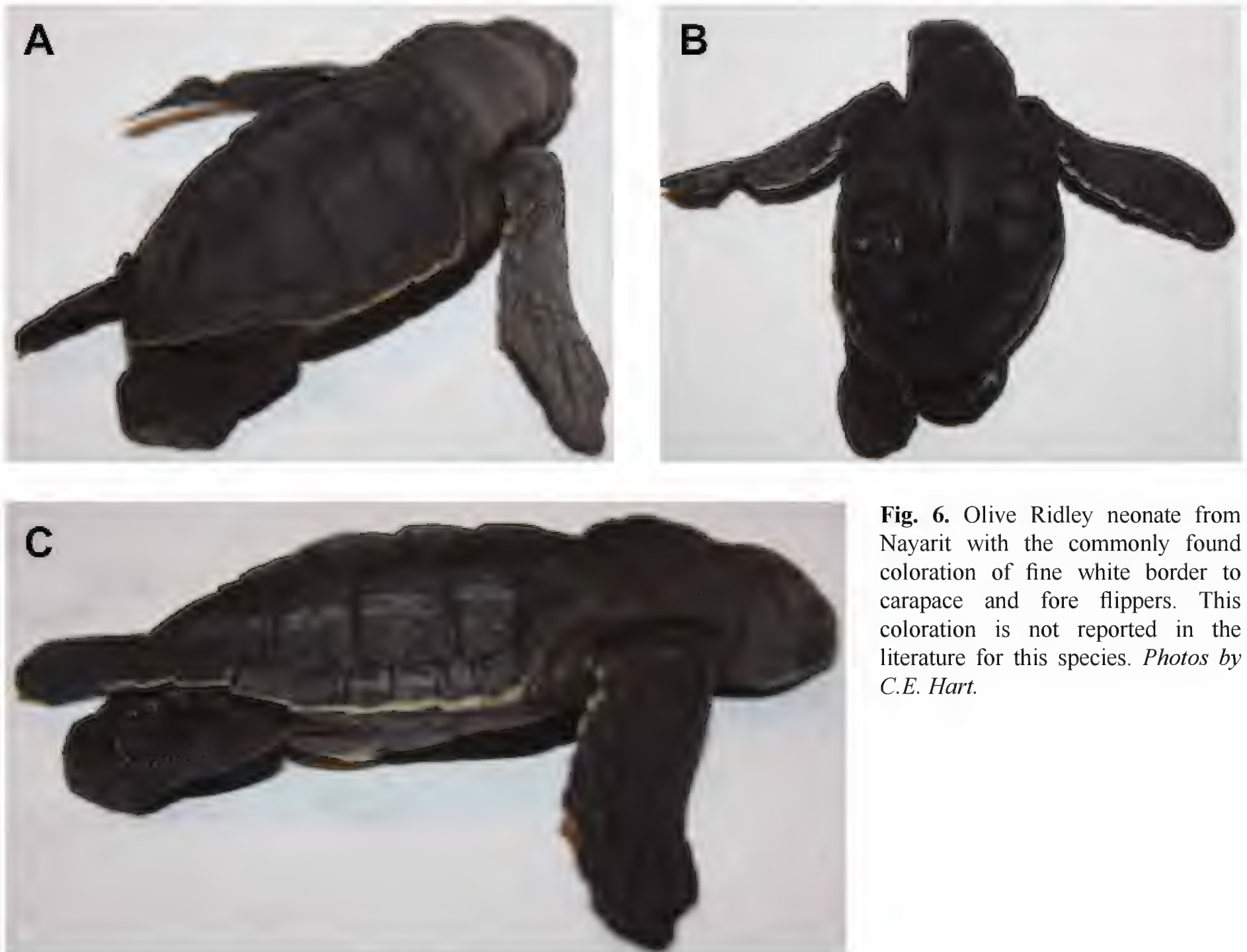


Fig. 6. Olive Ridley neonate from Nayarit with the commonly found coloration of fine white border to carapace and fore flippers. This coloration is not reported in the literature for this species. *Photos by C.E. Hart.*

Discussion

Olive Ridley turtles are the most abundant of all sea turtle species, and this is particularly evident in the East Pacific, with an estimated 1.39 million large juvenile and adult Olive Ridley turtles in the Tropical East Pacific (Eguchi et al. 2007). Because the vast differences in abundance would greatly increase the opportunities for hybridization of Olive Ridges with East Pacific Green Turtles, there is concern that if these become widespread they could jeopardize the recovery of the much smaller East Pacific Green Turtle population. For example, hybrids could be sterile and so reduce overall fertility. Hawksbill-Loggerhead hybrids have been found to be fertile in Brazil where introgression (breeding of hybrids with one or both parental taxa) has become significant (Lara-Ruiz et al. 2006). Furthermore, Soares et al. (2017) found that Loggerhead-Hawksbill hybrids were at no reproductive disadvantage relative to the pure Hawksbills among which they nested, suggesting that these hybrids are likely to persist there.

Many studies report either sterility or low fitness in hybrids (Allendorf et al. 2001). However, reports from conservation projects in northwest Mexico suggest that putative Olive Ridley-Green Turtle hybrid hatchlings are

more fit (being first to the water on release) than the Olive Ridley turtles that hatch from the same and/or different nests. However, the report of a more aggressive behavior by some of these hybrid hatchlings is also worrying.

We recommend using these illustrations to aid a national program aimed at compiling information on the frequency and locations of occurrence of atypical hatchlings within conservation projects, in order to gauge the importance of this phenomenon, coupled with genetic analyses to definitively confirm hybridization. If confirmed hybridization is found to be abundant, possible impacts on the regional populations are a cause for concern. We hope this information will open the conversation on the issue of hybridization between sea turtle species in Mexico and highlight the need for appropriate management guidelines to advise conservation projects on the action to take (if any) when these neonates occur.

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Literature Cited

- Allendorf FW, Leary RF, Spruell P, Wenburg JK. 2001. The problems with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution* 16(11): 613–622.
- Alvarado-Diaz J, Arias-Coyotl E, Delgado-Trejo C. 2003. Clutch frequency of the Michoacan Green Sea Turtle. *Journal of Herpetology* 37: 183–185.
- Arnold ML. 1997. *Natural Hybridization and Evolution*. Oxford University Press, Oxford, United Kingdom. 215 p.
- Ballorain K, Ciccione S, Bourjea J, Grizel H, Enstipp M, Georges JY. 2010. Habitat use of a multispecific seagrass meadow by Green Turtles *Chelonia mydas* at Mayotte Island. *Marine Biology* 157: 2,581–2,590.
- Barber RC, Fontaine CT, Flanagan JP, Louis EE Jr. 2003. Natural hybridization between a Kemps Ridley (*Lepidochelys kempii*) and Loggerhead Sea Turtle (*Caretta caretta*) confirmed by molecular analysis. *Chelonian Conservation and Biology* 4(3): 701–704.
- Bowen BW, Nelson WS, Avise JC. 1993. A molecular phylogeny for marine turtles: trait mapping, rate assessment, and conservation relevance. *Proceedings of the National Academy of Sciences of the United States of America* 90: 5,574–5,577.
- Chassin-Noria O, Abreu-Grobois A, Dutton PH, Oyama K. 2004. Conservation genetics of the East Pacific Green Turtle (*Chelonia mydas*) in Michoacan, Mexico. *Genetica* 121: 195–206.
- Dutton PH, Davis SK, Guerra T, Owens D. 1996. Molecular phylogeny for marine turtles based on sequences of the ND4-leucine tRNA and control regions of mitochondrial DNA. *Molecular Phylogenetics and Evolution* 5: 511–521.
- Eguchi T, Gerrodette T, Pitman RL, Seminoff JA, Dutton PH. 2007. At-sea density and abundance estimates of the Olive Ridley Turtle *Lepidochelys olivacea* in the eastern tropical Pacific. *Endangered Species Research* 3: 191–203.
- Espinosa-Carreón TL, Valez-Holguín JE. 2007. Gulf of California interannual chlorophyll variability. *Ecología Aplicada* 6(1–2): 83–92.
- García A, Ceballos G, Adaya R. 2003. Intensive beach management as an improved sea turtle conservation strategy in Mexico. *Biological Conservation* 111: 253–261.
- Genovart M. 2009. Natural hybridization and conservation. *Biodiversity Conservation* 18: 1,435–1,439.
- James MC, Martin K, Dutton PH. 2004. Hybridization between a Green Turtle, *Chelonia mydas*, and Loggerhead Turtle, *Caretta caretta*, and the first record of a Green Turtle in Atlantic Canada. *The Canadian Field-Naturalist* 118: 579–582.
- Jones MEH, Werneburg I, Curtis N, Penrose R, O'Higgins P, Fagan MJ, Evans SE. 2012. The head and neck anatomy of sea turtles (Cryptodira: Chelonioidae) and skull shape in Testudines. *PLoS One* 7: e47852.
- Karl SA, Bowen BW, Avise JC. 1995. Hybridization among the ancient mariners: characterization of marine turtle hybrids with molecular genetic assays. *Journal of Heredity* 86: 262–268.
- Kelez, S, Velez-Zuazo X, Pacheco AS. 2016. First record of hybridization between Green *Chelonia mydas* and Hawksbill *Eretmochelys imbricata* sea turtles in the Southeast Pacific. *PeerJ* 4: e1712.
- Lara-Ruiz P, Lopez GG, Santos FR, Soares LS. 2006. Extensive hybridization in Hawksbill Turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mtDNA analyses. *Conservation Genetics* 7(5): 773–781.
- Márquez R, Jiménez MC, Carrasco MA, Villanueva NA. 1998. Comentarios acerca de las tendencias poblacionales de las tortugas marinas del género *Lepidochelys* sp. Después de la veda total de 1990. *Océánides* 13(1): 41–62.
- Seminoff JA, Karl SA, Schwartz T, Resendiz A. 2003. Hybridization of the Green Turtle (*Chelonia mydas*) and Hawksbill Turtle (*Eretmochelys imbricata*) in the Pacific Ocean: indication of an absence of gender bias in the directionality of crosses. *Bulletin of Marine Science* 73: 643–652.
- Soares LS, Bolten AB, Wayne ML, Vilaça ST, Santos FR, dei Marcovaldi MAG, Bjorndal KA. 2017. Comparison of reproductive output of hybrid sea turtles and parental species. *Marine Biology* 164: 9.
- Tomas J, Aznar FJ, Raga JA. 2001. Feeding ecology of the Loggerhead Turtle *Caretta caretta* in the western Mediterranean. *Journal of Zoology* 255: 525–532.
- Wyneken J. 2001. *The Anatomy of Sea Turtles*. NOAA Technical Memorandum NMFS-SEFSC-470. United States Department of Commerce, Washington, DC, USA. 172 p.



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Diversity and conservation of terrestrial, freshwater, and marine reptiles and amphibians in Saudi Arabia

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Abstract.—This review describes the diversity of the freshwater, marine, and terrestrial herpetofauna of the Kingdom of Saudi Arabia that consists of 128 extant species and subspecies; 121 species and subspecies of reptiles and seven species of amphibians according to current taxonomic systems. Four main categories of threats affecting amphibians and reptiles were identified as habitat loss and degradation, water issues, human disturbance and related activities, and legislation and public awareness; and supportive examples for each category are provided. Key species that require urgent protection are: *Chalcides levitoni*, *Platycephalus insulanus*, *Dasypeltis scabra*, *Hemidactylus alfarraji*, *Hemidactylus asirensis*, *Hemidactylus mindiae*, *Lytorhynchus gasperetti*, *Pelomedusa barbata*, *Phoenicolacerta kulzeri* ssp., *Tropicolotes wolfgangboehmei*, and *Varanus yemenensis*, due to their limited distribution, as well as *Uromastix aegyptia* due to over-harvesting and trade. According to the IUCN Red List, eight of these species are Data Deficient, four are Vulnerable, one Critically Endangered, and one Near Threatened. The status of herpetofauna in Saudi Arabia is still far from being completely understood. Nevertheless, the lack of formal conservation measures and low public concern makes amphibians and reptiles extremely vulnerable in the near future.

Keywords. Anura, endemic, Sauria, Squamata, Testudines, threats

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Introduction

Over the past century, several major publications on the herpetofauna of Saudi Arabia have appeared (Schmidt 1941; Haas 1957; Haas and Werner 1969; Al-Wailly and Al-Uthman 1971; Farag and Banaja 1980; Hillenius and Gasperetti 1984; Balletto et al. 1985; Arnold 1986; Al-Sadoon 1988, 1989; Gasperetti 1988; Al-Sadoon et al. 1991; Leviton et al. 1992; Gasperetti et al. 1993; Schätti and Gasperetti 1994; Al-Johany 1995). Many additional publications have provided distributional data, taxonomic reviews, descriptions of new species, and new records (Hussein and Darwish 2001; Wilms and Böhme 2007; Al-Sadoon 2010; Cunningham 2010; Al-Shammari 2012; Šmíd et al. 2013, 2016; Al-Johany et al. 2014; Aloufi and Amr 2015; Alshammari and Ibrahim 2015; Al-Sadoon et al. 2016; Alshammari et al. 2017; Alqahtani 2018; Sindaco et al. 2018).

Some of these papers presented erroneous records that should be interpreted with caution. For example, Schätti and Gasperetti (1994) suggested that records of *Tarentola*

muritanica [sic] and *Tarentola annularis* by Farag and Banaja (1980) should be referred to as *Hemidactylus flaviviridis*. Also, the record of *Mabuya quinquetaeniata* from date gardens north of Umluj by Farag and Banaja (1980) certainly refers to *Trachylepis brevicollis*. Dekinesh (1991) included records of *Mabuya vittata*, *Stenodactylus petrii*, *Trapelus savignyi*, and *Sphenops sepsoides* from Faid Hema, which later proved to be misidentifications. Some of the contributions of Masood (2012) and Masood and Asiry (2012) to the herpetofauna of the Asir region comprise obvious misidentifications and erroneous records that call for amendments. For example, Masood and Asiry (2012) reported *T. annularis*, *T. mauritanica*, *Tropicolotes tripolitanus*, and *Trachylepis vittata* from the Asir region. Considering the known distribution ranges of the aforementioned species (e.g., see Schleich et al. 1996), these *Tarentola* records must be doubted as well. Other doubtful records include those of *Stenodactylus sthenodactylus*, *Trapelus pallidus*, and *Trapelus mutabilis* (Masood and Asiry 2012).

The conservation status of the terrestrial reptiles

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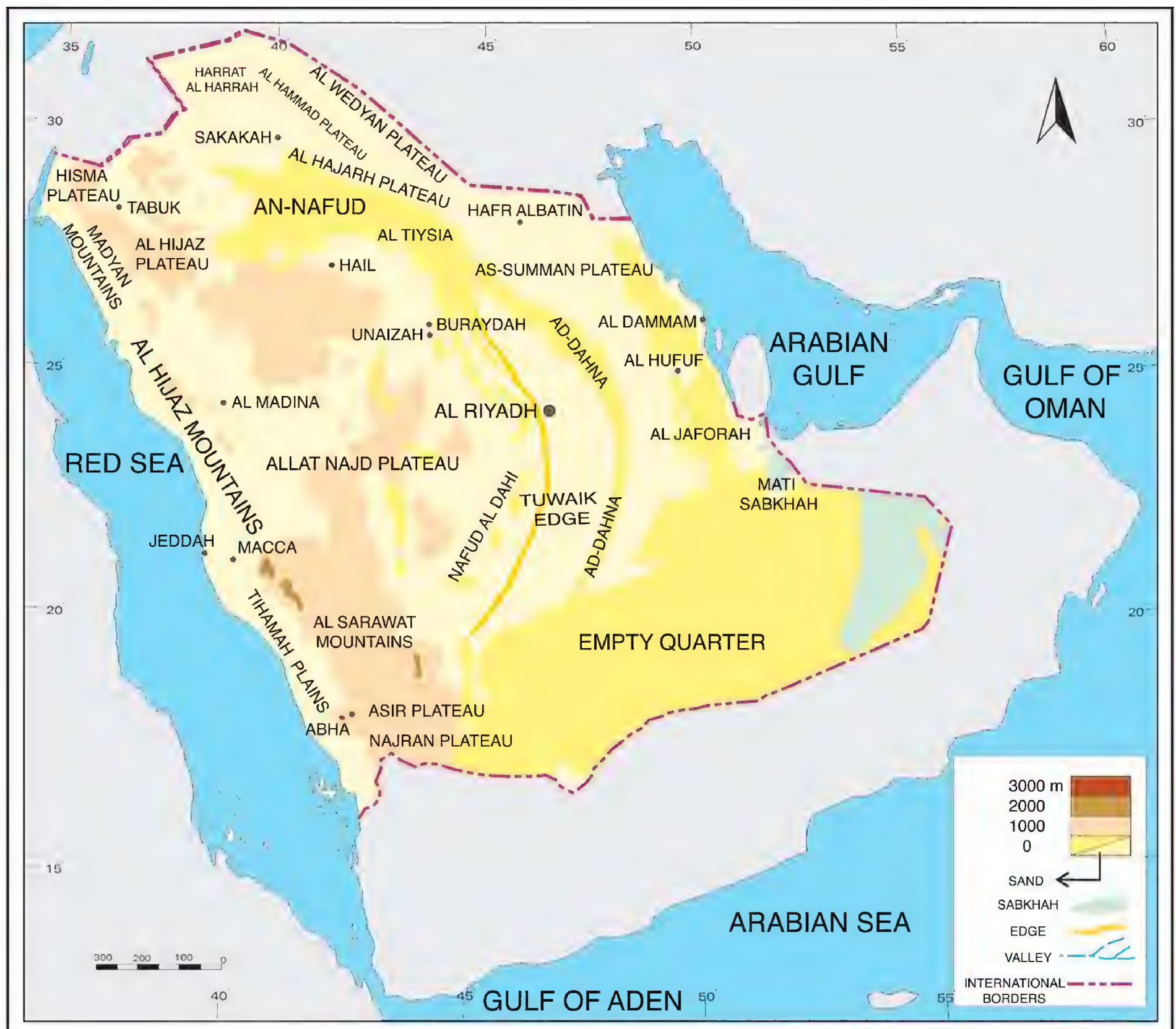


Fig. 1. Map of Saudi Arabia showing main geographic landmarks (after Al-Nafie 2018).

of the Arabian Peninsula was assessed by Cox et al. (2012). Some more recent works have focused on individual countries. Carranza et al. (2018) discussed the diversity, distribution, and conservation of the reptiles of Oman, including records for 101 species of terrestrial reptiles. The distribution of reptiles in Qatar was presented by Cogălniceanu et al. (2014). Gardner (2013) compiled distribution data for the amphibians and reptiles of Oman and the United Arab Emirates. Disi et al. (2014) gave a comprehensive account of the diversity, conservation, and major threats for the herpetofauna of Jordan.

Since the publications of Arnold (1986) on the lizards of Arabia, Gasperetti (1988) on the snakes of Arabia, and Leviton et al. (1992) on herpetofauna of the Middle East, no updated lists covering Saudi Arabia have been published. Many reptilian and amphibian species have been subjected to critical reviews on the molecular and morphological levels which resulted in the adoption of new names. In addition to documenting and updating the herpetofauna, based on current taxonomic understanding,

it is important to examine the impacts of continuous drastic changes in habitats and some practices in Saudi Arabia, as the reptiles and amphibians are being subjected to several forms of threats that have caused declines in some species.

In this study, the diversity, conservation status, and major threats affecting the herpetofauna of Saudi Arabia are identified, and an updated list, including the up-to-date taxonomic names, is provided.

Materials and Methods

The Approach

Scientific names of the reptilian species mostly follow Uetz et al. (2018). The taxonomic treatment of the genus *Phrynocephalus* by Melnikov et al. (2014) was adopted. The systematic position of *Trapelus ruderatus* (formerly *Trapelus persicus*) was followed after Ananjeva et al. (2013). For amphibians, the genera listed in the Amphibian Species of the World (<http://research.amnh>).



Fig. 2. Ad Disah mountains, southwest Tabuk. *Photo by S. Al Jathli.*

org/vz/herpetology/amphibia/) were adopted. The conservation status for species follow Cox et al. (2012). Data on the threats affecting the reptiles and amphibians were compiled from field observations mainly made by the first author, A. Aloufi. Distributional limits and localities were checked according to Sindaco and Jeremčenko (2008) for lizards, and Sindaco et al. (2013) for snakes.

Geographical Setting

Saudi Arabia is a vast country occupying 2,026,213 km² with diverse habitats that range from extreme arid and basalt deserts, to mountain ranges and highlands, sand and sandstone deserts, marine and freshwater ecosystems, and numerous wadi systems and oases (Figs. 1–3). Below is a general description of the geography of the Kingdom of Saudi Arabia, including the Coastal Plains, the Western Highlands, various plateaus, and sand dunes.

A. Coastal Plains

In Saudi Arabia, two strips of coastal plains extend along the Red Sea and the Arabian Gulf.

The coastal plain of the Red Sea (Tihammah Plains) lies between the Red Sea coast to the west and the western highlands to the east. It is a narrow transitional area between the Red Sea shelf and the high shelf mountains, which becomes wider in the south; reaching up to 40 km wide near Jazan. To the north, it becomes narrower until it disappears near 26°N latitude, south of Al Wajah. With the exception of some parts near the northern tip of the Red Sea, it is characterized by an abundance of capes. The northern half of the plain is characterized by a multitude of marine crusts, forming small marine

clefts that deepen inland by water flow descending from the coastal mountains such as Rabigh and Yanba'. The southern half is characterized by the spread of salt flats (*Sabkha*), especially along the coast, in addition to some sandy settlements near the coast, and the spread of some small black lava areas.

The coastal plain of the Arabian Gulf is limited to the gulf coast to the east, and the As-Summan plateau to the west. It is a flat plain largely covered by sand and salt flats (*Sabkha*), especially near the coast and along its side. This plain is devoid of wadi systems, with numerous sea extensions and capes. The lowest point in Saudi Arabia (24 m asl) lies within its southern part, near Al Homor Sabkha.

B. Western Highlands

The western highlands consist of a mountain chain that extends along the coast of the Gulf of Aqaba and the Red Sea, stretching from Jordan to the north and reaching the Republic of Yemen to the south. These mountains are a refractive ladder-shaped formation, and its western slopes descend precipitously towards the Red Sea, while inland they descend gradually eastward. The altitude increases towards the south, reaching as high as 3,015 m asl at the mountain of Al-Sūda, northwest of Abha. The western highlands are divided into three main mountain series: Al Sarawat mountains in the south, Al Hijaz mountains in the middle, and Madyan mountains in the north (Fig. 2).

C. Plateaus

The hills or plateaus located to the east of the western highlands cover large areas, and they generally descend to the east and north-east.

1. Western Plateaus

Najran and Asir plateaus are located to the east of the Sarawat mountains (Figs. 3A–B), between the Kingdom's borders and the Republic of Yemen to the south, and the drainage system of Wadi Al-Dawasir valley to the north. In this area of overlap they form a transitional area between the Sarawat Mountains in the west and the Empty Quarter in the east. Their altitudes range between 900 and 1,700 m asl.

The Hijaz Plateau is located to the east of the Hijaz Mountains, and is bounded by the Hijaz mountain to the west, the Najd Plateau to the east, the plateaus of Najran and Asir to the south, and the Hisma Plateau to the north. Its southern part descends to the north and west, and its northern part descends to the east and north-east. The altitude reaches up to 1,200 m asl. Some of its stretches include the black lava desert of Khaybar.

Hisam Plateau is located to the north-west of the Kingdom, to the east of the mountains of Medyan, and to the west and north-west of Tabuk. It is confined between the border with Jordan to the north, and Hijaz Plateau to the south. It descends eastwards, and ranges from 800–1,700 m asl. Black lava deserts cover some of its southern parts (Fig. 2).

2. Central Plateaus

The Central Plateau is represented by An-Nafud or Najd plateau, a large plateau located east of the Hijaz Plateau. It is bordered to the west by Hijaz Plateau, to the east by the sands of Ad-Dahna, to the south by the Wadi al-Dawasir, and by the Greater An-Nafud to the north. The An-Nafud near Tayma lies within the Arabian shield and is called Najd High Plateau, while the eastern part, known as Lower Najd Plateau, lies within the Arabian shelf. It gradually descends towards the north-east in the north, to the east in the middle section, and to the south-east in the south, with an average altitude of 500–1,000 m asl. The high plateau is characterized by igneous and metamorphic rocks. In contrast, the lower Najd Plateau is characterized by sand and rocky edges that extend from the north near Zulfi to the center of the southwestern extremities of the Empty Quarter, with a length of about 1,200 km.

3. Eastern Plateaus

The Eastern Plateaus are located in the eastern part of the Kingdom, and the extent from its center to its north-eastern edges is all within the Arabian shelf. The Eastern Plateaus include four main plateaus.

As-Summan Plateau is located to the east of Najd Plateau, where it is separated from Najd by Nofud Al Dahna, and extends from the north to the south. It is inserted between Ad-Dahna in the west, the coastal plains of the Arabian Gulf in the east, and from Yibreen to the

south of Wadi al-Sahba in the south. This plateau is wide and semi-flat, descending slightly towards the north-east and east, with altitudes ranging from 50–400 m asl.

Al Hajarh Plateau is located north-east of the Kingdom, between As-Summan Plateau in the south and Al-Hammad Plateau in the north. It is confined between the course of Wadi Al-Batin in the south, the Valley of Aba Al-Qor in the north, between the Nafud Al Dahna and the Greater Dahna in the west, and the border of the Kingdom of Saudi Arabia with Iraq in the east. A section of Al Hajarh also extends north of the Greater An-Nafud towards the west, reaching Al-Jawf, and it then descends towards the east and north-east, with altitudes ranging from 400–600 m asl.

Al Hammad Plateau is located in the north-east of the Kingdom, and it is confined between the Al Hajarh plateau in the south, the borders of the Kingdom with Iraq in the north, and between Harrat Al Harrah in the west (Fig. 3C), and Al Wedyan Plateau in the east. It descends to the northeast with altitudes ranging from 750–850 m asl.

Al Wedyan Plateau is located in the far northeast of the country, bordering Al Hammad plateau in the east, and it is considered as an extension of Al Hammad Plateau, descending towards the north-east, with altitudes ranging from 500–750 m asl. It is crossed by several wadi systems that descend the Al Hammad plateau and drain rainwater to Iraq during the rainy season.

D. Sand Dunes

The sand dunes cover a large proportion of the Kingdom's area, about 677,715 km², or about 33.8% of its total area. The sands of the Empty Quarter, Greater Nafud (Fig. 3E–F), and Al Jaforah are the largest sandy seas, together representing about 90% of the sand dunes in the country. The sand dunes are concentrated in the eastern part of the Kingdom, while small sandy gatherings occur in the Arabian shield to the west, in addition to small and scattered dunes along the Red Sea coast, formed from the presence of sediment sources, watercourses, and wind.

Biogeographical regions of Saudi Arabia

Al-Nafie (2008) defined four main phytogeographical regions in the Kingdom of Saudi Arabia (Fig. 4). The Saharo-Arabian region occupies the greatest part of Saudi Arabia, extending from the north, through central Arabia. It includes As-Summan, Al Hammad, Al Hajarh, Al Wedyan, and Najd plateaus, An-Nafud, Ad-Dahna, and the Empty Quarter sand dunes. The Afromontane region has mountains higher than 1,800 m asl, is dominated by *Juniperus procera* and other evergreen shrubs, and covers a narrow strip extending along Asir and Sarawat mountains. The Sudanian region stretches over a narrow

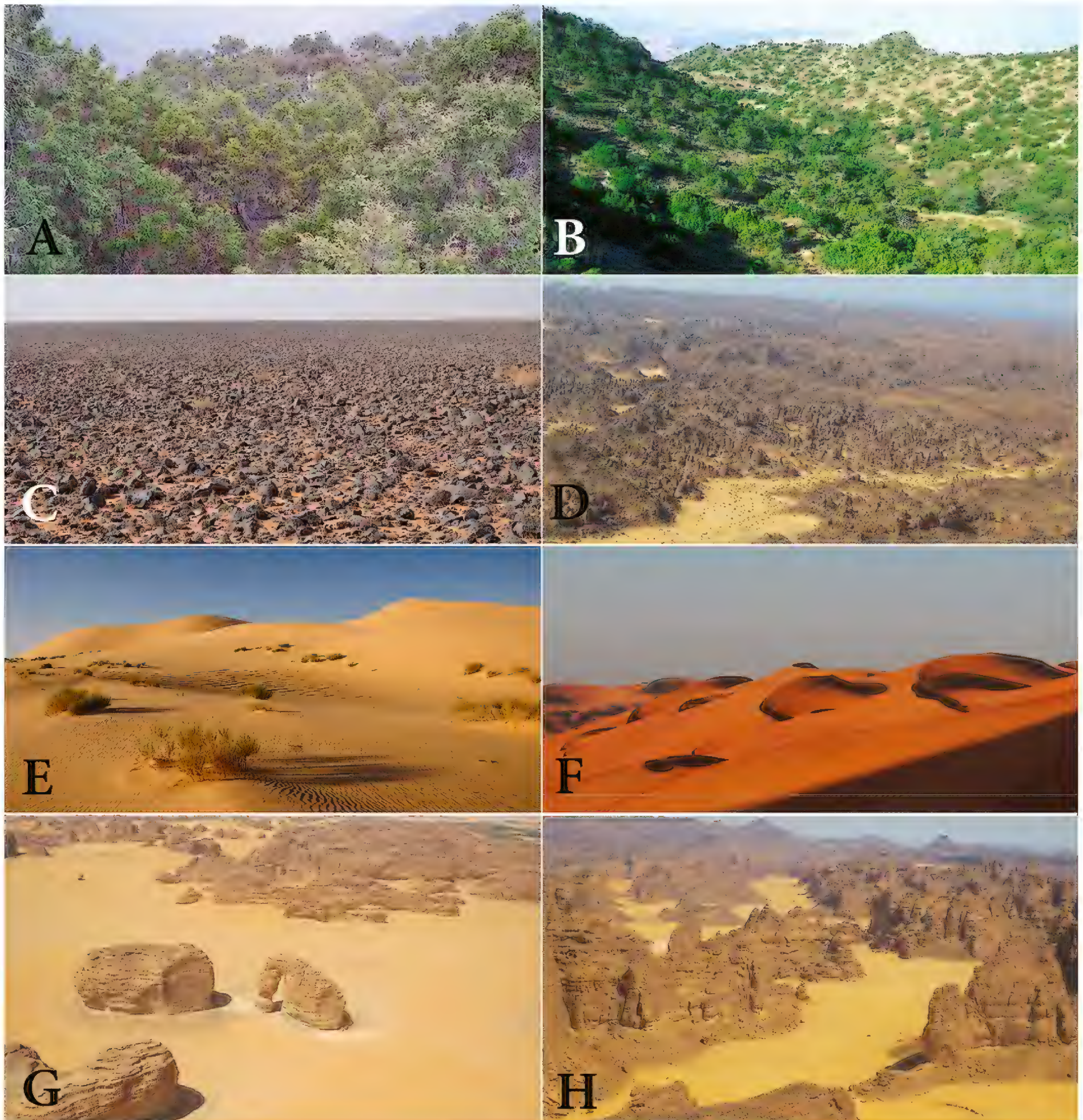


Fig. 3. Landscapes and habitats in Saudi Arabia. **(A)** *Juniperus procera* forests in Raydah reserve. **(B)** *Juniperus procera* forests in Asir mountains. **(C)** Harrat Al Harrah. **(D)** Harat Ewardh. **(E)** Sand dunes in the Greater Nofoud. **(F)** Sand dunes in the Empty Quarter. **(G)** Elephant mountain in Al-'Ula. **(H)** Sharaan sand stones mountains in Al-'Ula. Photos by K. Al Shamari (A), O. Llewellyn (B), and A. Aloufi (C–H).

strip along the Red Sea coast as well as the Arabian Gulf coast. Finally, the Sudanian-Zambian region surrounds the Afromontane region, with overlaps with the Sudanian region along the southwestern portions.

Results

Amphibians

Balletto et al. (1985) gave the most comprehensive treatment to date of the amphibians of Arabia. Additional distribution data were presented for central (Al-Johany

2014) and southwestern Saudi Arabia (Al-Qahtani and Al-Johany 2018), by Schätti and Gasperetti (1994) for southwest Arabia, and by Alshammari and Ibnrahim (2018) for Hai'l. Recent studies replaced *Hyla savignyi* with the newly described taxon *Hyla felixarabica* (Gvožik et al. 2010). Previous records of *Bufotes viridis* are now considered as *Bufotes boulengeri* (see <http://research.amnh.org/vz/herpetology/amphibia/>). In total, seven species belonging to four families (Bufonidae, Ranidae, Hylidae, and Dicroglossidae) are known from the Kingdom of Saudi Arabia (Fig. 5, Table 1).

Diversity and conservation of reptiles and amphibians in Saudi Arabia

Table 1. List of amphibians and reptiles of Saudi Arabia, their IUCN conservation status, and levels of threats. IUCN status: Critically Endangered (CR); Data Deficient (DD); Endangered (EN); Least Concern (LC); Near Threatened (NT); Not Evaluated (NE); Vulnerable (VU). Threats: 1 = Deforestation, 2 = Destruction of the natural vegetation in the desert, 3 = Agricultural expansion, 4 = Overgrazing, 5 = Water extraction and climate change, 6 = Pollution and marine debris, 7 = Recreational activities and tourism, 8 = Direct persecution, 9 = Trade and commercial collection, 10 = Hunting and poaching. Levels of threats: L = Low, M = Medium, H = High.

	IUCN status		Threats									
	Global	Regional	1	2	3	4	5	6	7	8	9	10
Amphibia												
Family Bufonidae												
<i>Bufotes boulengeri</i> (Lataste, 1879)	LC	NE					L				L	
<i>Duttaphrynus dhufarensis</i> (Parker, 1931)	LC	NE					M				L	
<i>Sclerophrys arabica</i> (Heyden, 1827)	LC	NE					M				L	
<i>Sclerophrys tihamica</i> (Balletto and Cherchi, 1973)	LC	NE					M				L	
Family Ranidae												
<i>Pelophylax ridibundus</i> (Pallas, 1771)	LC	NE					M				L	
Family Hylidae												
<i>Hyla felixarabica</i> Gvoždík et al. 2010	NE	NE					M				L	
Family Dicroglossidae												
<i>Euphlyctis ehrenbergii</i> (Peters, 1863)	LC	NE					M				L	
Reptilia												
Family Geoemydidae												
<i>Mauremys caspica</i> (Gmelin, 1774)	LC	VU					H				L	
Family Pelomedusidae												
<i>Pelomedusa barbata</i> Petzold et al. 2014	NE	NE	M				H				L	
Family Cheloniidae												
<i>Eretmochelys imbricata</i> (Linnaeus, 1766)	CR	NE						L			L	
<i>Chelonia mydas</i> (Linnaeus, 1758)	EN	NE						H			L	
<i>Caretta caretta</i> (Linnaeus, 1758)	VU	NE						L			L	
<i>Lepidochelys olivacea</i> (Eschscholtz, 1829)	VU	NE						L			L	
Family Dermochelyidae												
<i>Dermochelys coriacea</i> (Vandelli, 1761)	VU	NE						L			L	
Family Chamaeleonidae												
<i>Chamaeleo calypttratus calcarifer</i> Peters 1871	LC	LC	M								L	
<i>Chamaeleo chamaeleon orientalis</i> Parker 1938	LC	LC		L							L	
Family Agamidae												
<i>Acanthocercus adramitanus</i> (Anderson, 1896)	LC	LC	L						L			
<i>Acanthocercus yemenensis</i> (Klausewitz, 1954)	LC	LC	L						L			
<i>Phrynocephalus arabicus</i> Anderson, 1984	LC	LC			L				L			
<i>Phrynocephalus nejdensis</i> Haas, 1957	LC	LC			L				L			
<i>Phrynocephalus macropeltis</i> Melnikov et al. 2014	NE	NE			L				L			
<i>Phrynocephalus maculatus</i> Anderson, 1872	NE	NE			L				L			
<i>Pseudotrapelus sinaitus</i> Heyden, 1827	LC	LC				L			L			
<i>Pseudotrapelus aqabensis</i> Melnikov et al. 2012	NE	NE				L			L			
<i>Stellagama stellio</i> (Linnaeus, 1758)	LC	LC				L			L			
<i>Trapelus agnetae</i> (Werner, 1929)	LC	LC			L				L			
<i>Trapelus flavimaculatus</i> Rüppell, 1835	LC	LC			L				L			
<i>Trapelus jayakari</i> (Anderson, 1896)	DD	DD		L	L	L			L			
<i>Trapelus persicus</i> (Blanforld, 1804)	LC	LC		L		L			L			

Table 1 (continued). List of amphibians and reptiles of Saudi Arabia, their IUCN conservation status, and levels of threats. IUCN status: Critically Endangered (CR); Data Deficient (DD); Endangered (EN); Least Concern (LC); Near Threatened (NT); Not Evaluated (NE); Vulnerable (VU). Threats: 1 = Deforestation, 2 = Destruction of the natural vegetation in the desert, 3 = Agricultural expansion, 4 = Overgrazing, 5 = Water extraction and climate change, 6 = Pollution and marine debris, 7 = Recreational activities and tourism, 8 = Direct persecution, 9 = Trade and commercial collection, 10 = Hunting and poaching. Levels of threats: L = Low, M = Medium, H = High.

	IUCN status		Threats									
	Global	Regional	1	2	3	4	5	6	7	8	9	10
<i>Uromastix aegyptia</i> (Forskal, 1775)	VU	VU		H		H			L		H	H
<i>Uromastix ornata</i> Heyden, 1827	LC	LC		L		L			L			
Family Gekkonidae												
<i>Bunopus tuberculatus</i> Blanford, 1874	LC	LC			L							
<i>Cyrtopodion scabrum</i> (Heyden, 1827)	LC	LC				L			L	L		
<i>Hemidactylus alfarraji</i> Šmíd et al. 2016	NE	NE				L			L	L		
<i>Hemidactylus asirensis</i> Šmíd et al. 2016	NE	NE				L			L	L		
<i>Hemidactylus flaviviridis</i> Rüppell, 1835	LC	LC				L			L	L		
<i>Hemidactylus granosus</i> Heyden, 1827	NE	NE				L			L	L		
<i>Hemidactylus mendiae</i> Baha El Din, 2005	NE	NE				L			L	L		
<i>Hemidactylus montanus</i> Busais and Joger, 2011						L			L	L		
<i>Hemidactylus persicus</i> Anderson, 1872	LC	LC				L			L	L		
<i>Hemidactylus robustus</i> Heyden, 1827						L			L	L		
<i>Pseudoceramodactylus khobarensis</i> Haas, 1957	LC	LC		L	L				L	L		
<i>Stenodactylus doriae</i> (Blanford, 1874)	LC	LC		L	L				L	L		
<i>Stenodactylus grandiceps</i> Haas, 1952	LC	LC		L	L				L	L		
<i>Stenodactylus slevini</i> Haas, 1957	LC	LC		L	L				L	L		
<i>Stenodactylus yemenensis</i> Arnold, 1980	LC	LC		L	L				L	L		
<i>Trigonodactylus arabicus</i> (Haas, 1957)	LC	LC		L		L			L	L		
<i>Tropicolotes nattereri</i> Steindachner, 1901	LC	LC		L		L			L	L		
<i>Tropicolotes wolfgangboehmei</i> Wilms et al. 2010	DD	DD		L	L					L		
Family Phyllodactylidae												
<i>Ptyodactylus hasselquistii</i> (Donndorff, 1798)	LC	LC				L				L		
Family Sphaerodactylidae												
<i>Pristurus carteri</i> (Gray, 1863)	LC	LC				L			L	L		
<i>Pristurus flavipunctatus</i> Rüppell, 1835	LC	LC				L			L	L		
<i>Pristurus minimus</i> Arnold, 1977	LC	LC				L			L	L		
<i>Pristurus popovi</i> Arnold, 1982	LC	LC				L			L	L		
<i>Pristurus rupestris</i> Blanford, 1874	LC	LC				L			L	L		
Family Lacertidae												
<i>Acanthodactylus boskianus</i> (Daudin, 1802)	LC	LC		L	L	L			L			
<i>Acanthodactylus gongrorhynchatus</i> Leviton and Anderson, 1967	DD	DD		L	L				L			
<i>Acanthodactylus grandis</i> Boulenger, 1909	LC	LC		L	L	L			L			
<i>Acanthodactylus haasi</i> Leviton and Anderson, 1967	LC	LC		L	L	L			L			
<i>Acanthodactylus opheodurus</i> Arnold, 1980	LC	LC		L	L	L			L			
<i>Acanthodactylus robustus</i> (Werner, 1929)	LC	LC		L	L	L			L			
<i>Acanthodactylus schmidtii</i> Haas, 1957	LC	LC		L	L	L			L			
<i>Acanthodactylus scutellatus</i> (Audouin, 1827)	LC	LC		L	L	L			L			
<i>Acanthodactylus orientalis</i> Angel, 1936	LC	LC		L	L	L			L			
<i>Acanthodactylus tilburyi</i> Arnold, 1986	LC	LC		L	L	L			L			

Diversity and conservation of reptiles and amphibians in Saudi Arabia

Table 1 (continued). List of amphibians and reptiles of Saudi Arabia, their IUCN conservation status, and levels of threats. IUCN status: Critically Endangered (CR); Data Deficient (DD); Endangered (EN); Least Concern (LC); Near Threatened (NT); Not Evaluated (NE); Vulnerable (VU). Threats: 1 = Deforestation, 2 = Destruction of the natural vegetation in the desert, 3 = Agricultural expansion, 4 = Overgrazing, 5 = Water extraction and climate change, 6 = Pollution and marine debris, 7 = Recreational activities and tourism, 8 = Direct persecution, 9 = Trade and commercial collection, 10 = Hunting and poaching. Levels of threats: L = Low, M = Medium, H = High.

	IUCN status		Threats									
	Global	Regional	1	2	3	4	5	6	7	8	9	10
<i>Mesalina arnoldi</i> Sindaco et al., 2018	NE	NE							L			
<i>Mesalina adramitana</i> (Boulenger, 1917)	LC	LC		L	L				L			
<i>Mesalina bernoullii</i> (Schenkel, 1901)	NE	NE		L	L				L			
<i>Mesalina brevirostris</i> Blanford, 1874	LC	LC		L	L				L			
<i>Mesalina guttulata</i> (Lichtenstein, 1823)	LC	LC		L	L				L			
<i>Mesalina olivieri</i> (Audouin, 1829)	LC	LC		L	L				L			
<i>Mesalina saudiarabica</i> Moravec et al. 2017	NE	NE		L	L				L			
<i>Ophisops elbaensis</i> Schmidt and Marx, 1957	DD	DD		L	L				L			
<i>Philochortus neumanni</i> Matschie, 1893	LC	LC		L	L				L			
<i>Phoenicolacerta kulzeri</i> ssp.	EN	NE			L	L			L			
Family Scincidae												
<i>Ablepharus pannonicus</i> (Fitzinger, 1824)	LC	LC		L		L						
<i>Chalcides levitoni</i> Pasteur, 1978	DD	DD	L	L	L	L						
<i>Chalcides ocellatus</i> (Forskål, 1775)	LC	LC		L		L						
<i>Eumeces schneideri</i> (Daudin, 1802)	LC	LC		L		L						
<i>Eurylepis taeniolatus</i> Blyth, 1854	LC	LC			L							
<i>Scincus hemprichii</i> Wiegmann, 1837	LC	LC		L	L				L			
<i>Scincus mitranus</i> Anderson, 1871	LC	LC		L	L				L			
<i>Scincus scincus</i> (Linnaeus, 1758)	LC	LC		L	L				L			
<i>Trachylepis brevicollis</i> (Wiegmann, 1837)	LC	LC		L		L			L			
<i>Trachylepis septemtaeniatus</i> (Reuss, 1834)	LC	LC		L		L			L			
Family Trogonophidae												
<i>Diplometopon zarudnyi</i> Nikolsky, 1907	LC	LC		L	L	L						
Family Varanidae												
<i>Varanus griseus</i> (Daudin, 1803)	LC	LC		L	L				L		M	
<i>Varanus yemenensis</i> Böhme et al. 1989	DD	DD		L	L				L		L	
Family Atractaspididae												
<i>Atractaspis andersonii</i> Boulenger, 1905	LC	LC								M		
<i>Atractaspis engaddensis</i> Haas, 1950	LC	LC								M		
Family Boidae												
<i>Eryx jaculus</i> Linnaeus, 1758	LC	LC		L	L				L		L	
<i>Eryx jayakari</i> Boulenger, 1888	LC	LC		L	L				L		L	
Family Colubridae												
<i>Dasypeltis scabra</i> (Linnaeus, 1758)	LC	LC	L			L						
<i>Eirenis coronella coronella</i> (Schlegel, 1837)	LC	LC		L	L							
<i>Eirenis coronella fennelli</i> Arnold, 1982	LC	LC		L	L							
<i>Lytorhynchus diadema</i> (Duméril et al. 1854)	LC	LC		L	L							
<i>Lytorhynchus gasperetti</i> Leviton, 1977	DD	DD			L	L						
<i>Platyiceps elagantissimus</i> (Günther, 1878)	LC	LC		L		L						
<i>Platyiceps insulanus</i> (Mertens, 1965)	DD	DD		L	L	L						
<i>Platyiceps rhodarchis</i> (Jan, 1865)	LC	LC		L		L					L	

Table 1 (continued). List of amphibians and reptiles of Saudi Arabia, their IUCN conservation status, and levels of threats. IUCN status: Critically Endangered (CR); Data Deficient (DD); Endangered (EN); Least Concern (LC); Near Threatened (NT); Not Evaluated (NE); Vulnerable (VU). Threats: 1 = Deforestation, 2 = Destruction of the natural vegetation in the desert, 3 = Agricultural expansion, 4 = Overgrazing, 5 = Water extraction and climate change, 6 = Pollution and marine debris, 7 = Recreational activities and tourism, 8 = Direct persecution, 9 = Trade and commercial collection, 10 = Hunting and poaching. Levels of threats: L = Low, M = Medium, H = High.

	IUCN status		Threats									
	Global	Regional	1	2	3	4	5	6	7	8	9	10
<i>Platyceps saharicus</i> Schätti and McCarthy, 2004	LC	LC		L		L						
<i>Platyceps variabilis</i> (Boulenger, 1905)	LC	LC		L		L						
<i>Platyceps ventromaculatus</i> (Gray, 1834)	LC	LC		L		L						
<i>Spalerosophis diadema cliffordii</i> (Schlegel, 1837)	LC	LC		L		L					L	
<i>Telescopus dhara</i> (Forsskål, 1775)	LC	LC		L	L	L						
Family Elapidae												
<i>Hydrophis curtus</i> (Shaw, 1802)	LC	NE						L				
<i>Hydrophis cyanocinctus</i> Daudin, 1803	LC	NE						L				
<i>Hydrophis lamberti</i> Smith, 1917	LC	NE						L				
<i>Hydrophis lapemoides</i> (Gray, 1849)	LC	NE						L				
<i>Hydrophis gracilis</i> (Shaw, 1802)	LC	NE						L				
<i>Hydrophis platurus</i> (Linnaeus, 1766)	LC	NE						L				
<i>Hydrophis schistosa</i> Daudin, 1803	LC	NE						L				
<i>Hydrophis spiralis</i> (Shaw, 1802)	LC	NE						L				
<i>Hydrophis viperinus</i> (Schmidt 1852)	LC	NE						L				
<i>Naja arabica</i> Scortecci, 1932	LC	LC							L	H	M	
<i>Walterinnesia aegyptia</i> Lataste, 1887	LC	LC								L		
<i>Walterinnesia morgani</i> (Mocquard, 1905)	VU	VU								L		
Family Leptotyphlopidae												
<i>Myriopholis macrorhyncha</i> (Jan, 1860)	LC	LC		L								
<i>Myriopholis nursii</i> (Anderson, 1896)	LC	LC		L								
Family Typhlopidae												
<i>Indotyphlops braminus</i> (Daudin, 1803)	LC	LC		L								
Family Psammophiidae												
<i>Psammophis schokari</i> (Forsskål, 1775)	LC	LC		L							M	
<i>Rhagerhis moilensis</i> (Reuss, 1834)	LC	LC		L						M		
Family Viperidae												
<i>Bitis arietans</i> Merrem, 1820	LC	LC								L	L	
<i>Cerastes cerastes</i> (Linnaeus, 1758)	LC	LC								L	L	
<i>Cerastes gasperetti</i> Leviton and Anderson, 1967	LC	LC								L	L	
<i>Echis borkini</i> Cherlin, 1990	LC	LC	L							L		
<i>Echis coloratus</i> Günther, 1878	LC	LC								L	L	
<i>Pseudocerastes fieldi</i> Schmidt, 1930	LC	LC								L		

Reptiles

species, have been recorded from Saudi Arabia (Table 1).

Twenty-one families in two orders, Testudines (Cheloniidae, Dermochelyidae, Geoemydidae, and Pelomedusidae) and Squamata (Agamidae, Atractaspididae, Boidae, Chamaeleonidae, Colubridae, Gekkonidae, Elapidae, Lacertidae, Leptotyphlopidae, Phyllodactylidae, Psammophiidae, Scincidae, Sphaerodactylidae, Trogonophidae, Typhlopidae, Varanidae, and Viperidae), including 55 genera and 121

Testudines

Two species of freshwater turtles belonging to two families have been recorded from Saudi Arabia: *Pelomedusa barbata* (Pelomedusidae, Petzold et al. 2014) and *Mauremys caspica* (Geoemydidae, Gasperetti et al. 1993; Vamberger et al. 2013). Alqahtani (2017) published a detailed account on the status and distribution

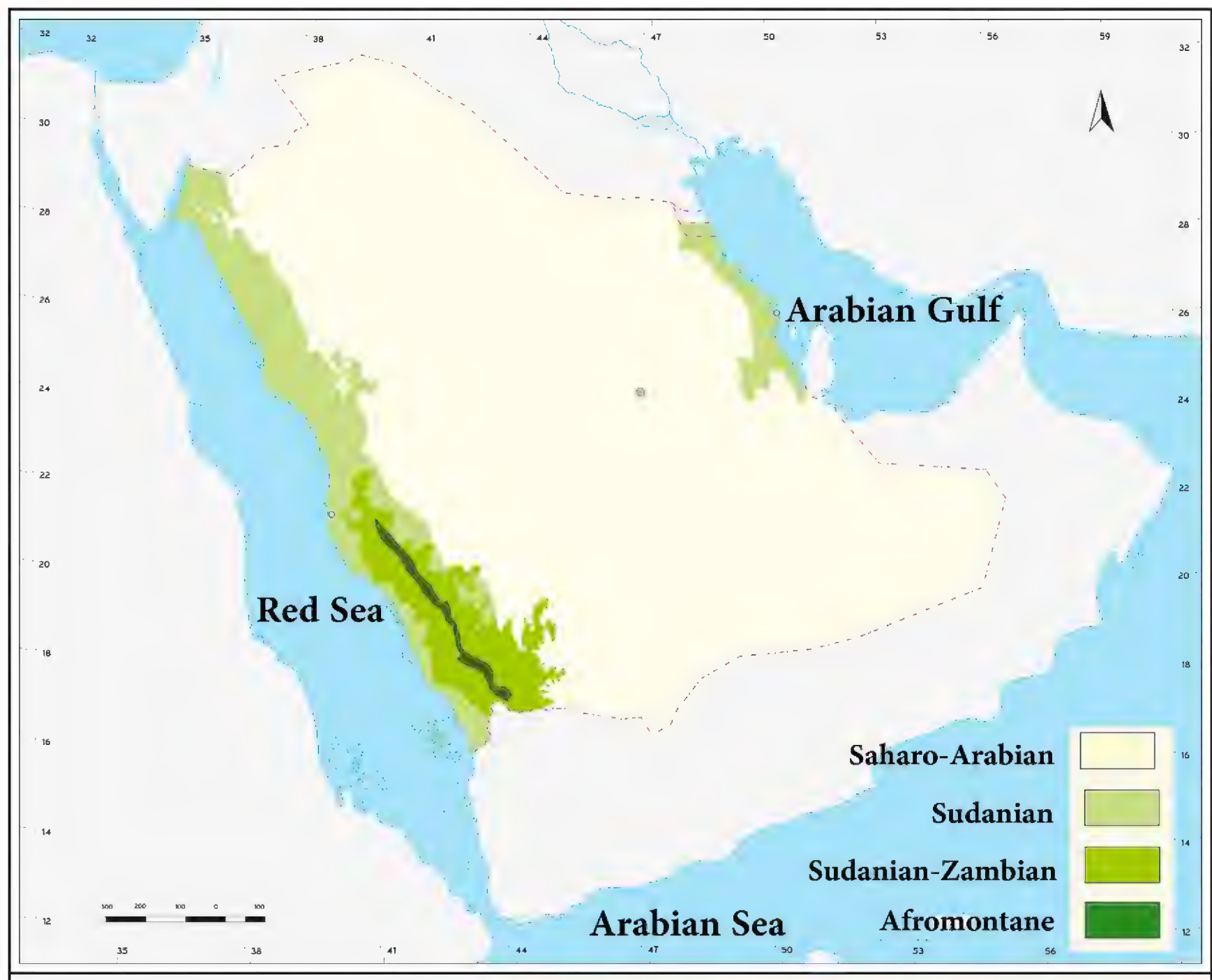


Fig. 4. Phytogeographical regions of Saudi Arabia (after Al-Nafie 2008).

of *P. subrufa* (= *Pelomedusa barbata*) in Saudi Arabia.

Five species of marine turtles in two families (Cheloniidae and Dermochelyidae) are known to occur in the Red Sea (Ross 1985; Gasperetti et al. 1993; Al-Merghani et al. 2000; Pilcher et al. 2014; Mancini et al. 2015). The Green Turtle, *Chelonia mydas*, and the Hawksbill Turtle, *Eretmochelys imbricata*, are considered as the most common species in the Red Sea (Fig. 6), where they feed and nest (PERSGA 2004).

Squamata

Family Chamaeleonidae

Hillenius and Gasperetti (1984) listed two taxa of chameleons in Saudi Arabia: *Chamaeleo calypttratus calcarifer* Peters 1871 and *Chamaeleo chamaeleon orientalis* Parker 1938. Both species are confined to the coastal mountains of the Red Sea, reaching up to 2,500 m asl.

Family Agamidae (Fig. 7)

Fifteen species belonging to six genera (*Acanthocercus*, *Phrynocephalus*, *Pseudotrapelus*, *Stellagama*, *Trapelus*, and *Uromastyx*) have been reported from Saudi Arabia.

The status of species of the genus *Phrynocephalus* were subjected to revisions based on the morphological and genetic differences. Melnikov et al. (2014) differentiated between the members of the *Phrynocephalus arabicus* Anderson, 1984 complex. Their study revealed that *Ph. arabicus sensu stricto* is distributed in southern Arabia (Yemen, Oman, southern Saudi Arabia), and *Ph. nejdensis* in north-western Arabia (southern Jordan, northern and central Saudi Arabia), while *Ph. macropeltis* is known in the eastern coastal Arabia (eastern Saudi Arabia, United Arab Emirates).

Rastegar-Pouyani (2000) published a controversial opinion on the identity of *Trapelus persicus*. He stated that the specific name “*runderatus*” (Olivier, 1804) antedates “*persicus*” (Blanford 1804), so the new taxonomic combination is *Trapelus ruderatus ruderatus* (Olivier, 1804) (= the former *T. p. persicus*), and the western subspecies *T. p. fieldi* has a new taxonomic combination. Therefore, the name “*persicus*” is no longer available and comes under the synonymy of “*runderatus*.” More recently, Ananjeva et al. (2013) clarified the systematic position of *Trapelus ruderatus* in relation to *T. persicus*, and for constancy and stability of the taxonomy of this group, the International Code of Zoological Nomenclature ruled that these two species are separate. In addition, *Pseudotrapelus aqabensis* was



Fig. 5. Amphibians of Saudi Arabia. (A) *Euphlyctis ehrenbergii*. (B) *Sclerophrys tihamica*. Photos by T. Papenfuss.

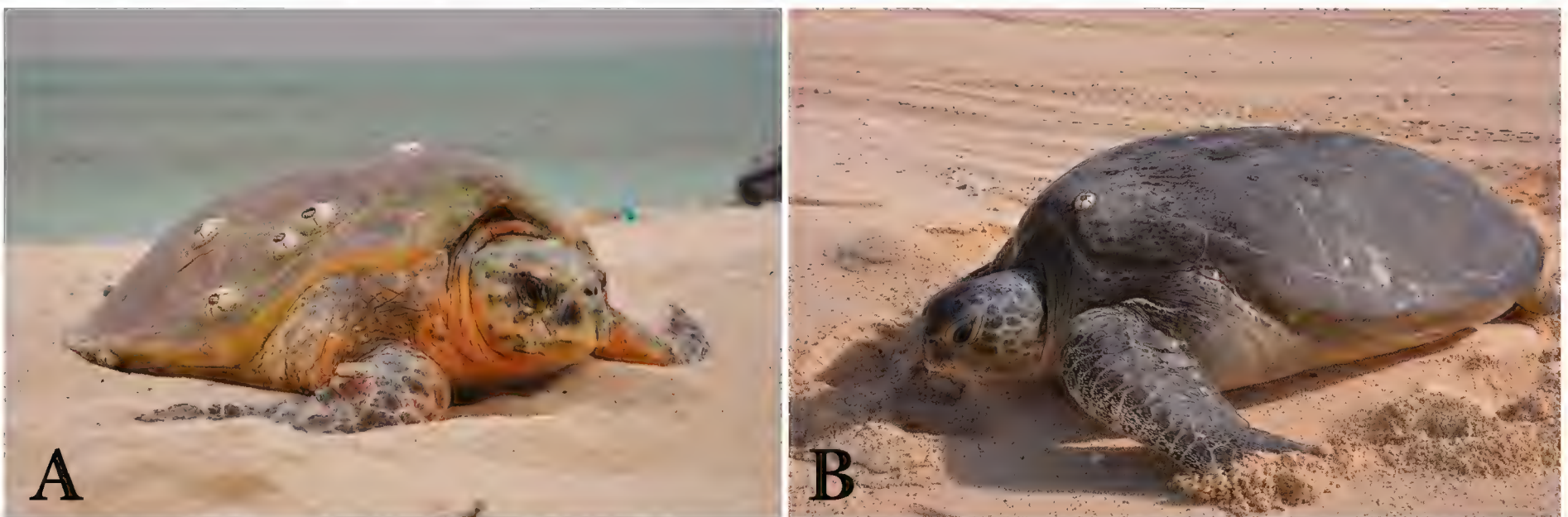


Fig. 6. Marine turtles of Saudi Arabia. (A) *Eretmochelys imbricata*. (B) *Chelonia mydas*. Photos by A. Al Mansi.



Fig. 7. Agamids of Saudi Arabia. (A) *Trapelus flavimaculatus*. (B) *Stellagama stellio*. (C) *Phrynocephalus nejdensis*. (D) *Pseudotrapelus sinaitus*. Photos by A. Aloufi.

added recently to the herpetofauna of Saudi Arabia by Aloufi and Amr (2015) and Tamar et al. (2016b).

Family Gekkonidae

This family is represented by seven genera (*Bunopus*,

Cyrtopodion, *Hemidactylus*, *Pseudoceramodactylus*, *Stenodactylus*, *Trigonodactylus*, and *Tropiocolotes*), including 18 species. The genus *Stenodactylus* was revised by Arnold (1980), Metallinou et al. (2012), and Nazarov et al. (2018), and it now includes four species. Šmíd et al. (2013) revised the genus *Hemidactylus*. They considered

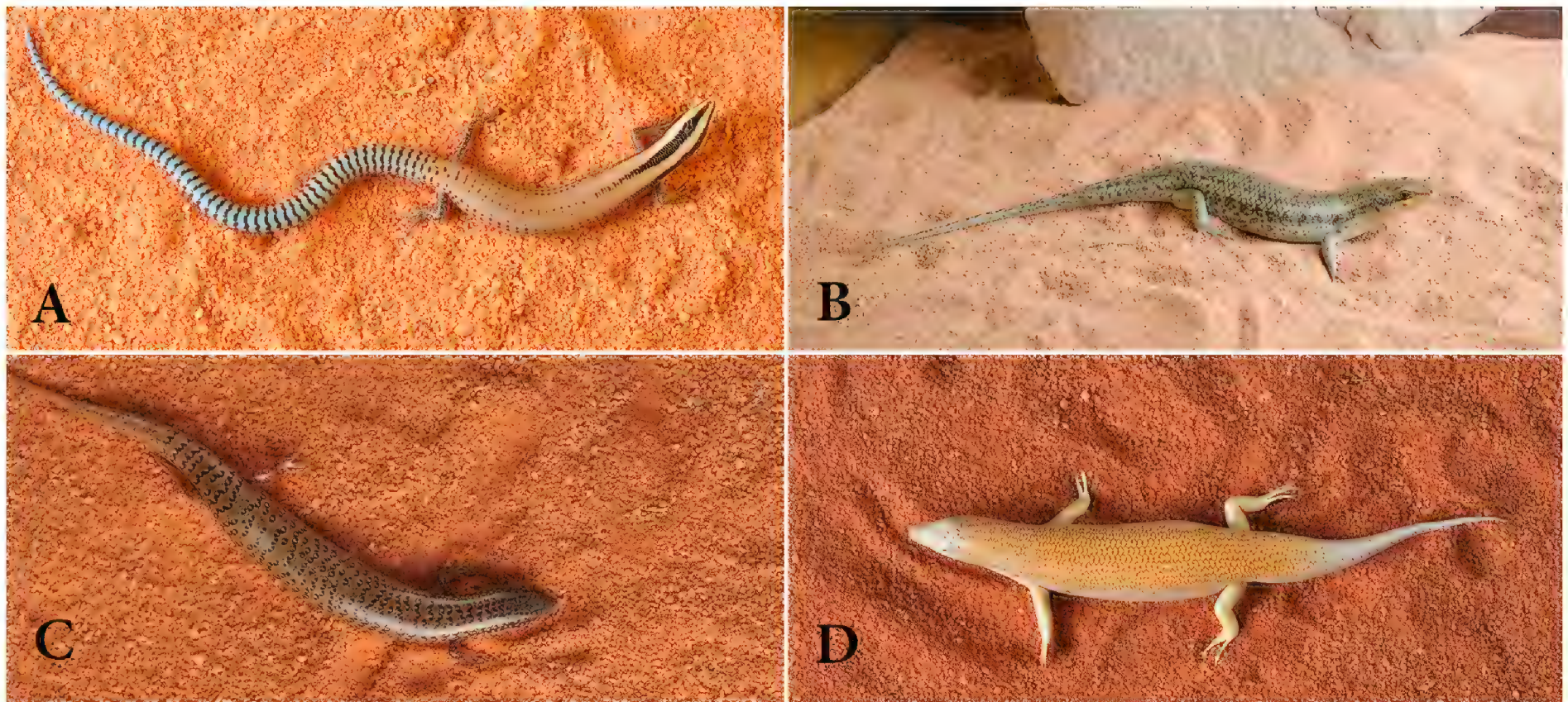


Fig. 8. Scincids of Saudi Arabia. (A) *Eurylepis taeniolatus*. (B) *Trachylepis brevicollis*. (C) *Chalcides ocellatus* (D) *Scincus scincus*. Photos by A. Aloufi.

all previous records of *Hemidactylus turcicus* from Saudi Arabia as *Hemidactylus granosus*. Recently Šmíd et al. (2016) described two endemic species for Saudi Arabia: *Hemidactylus alfarraji* from Najran area and *Hemidactylus asirensis* from Asir Province. *Hemidactylus mindiae* is a new addition to the geckos of Saudi Arabia (Aloufi and Amr 2015). Therefore, the number of *Hemidactylus* species known from Saudi Arabia is now eight. One record of *Hemidactylus sinaitus* Boulenger, 1885 from Seir Farasan Kebir should be considered with care (see Sindaco et al. 2014). *Tropicolotes wolfgangboehmei* is known from a single locality in central Saudi Arabia (Wilms et al. 2010).

Family Phyllodactylidae

This family is represented by one genus and one species, *Ptyodactylus hasselquistii*. Recent molecular studies revealed that the genus *Ptyodactylus* in the Arabian Peninsula consists of two species complexes for *P. hasselquistii*, as eastern and western clades (Metallinou et al. 2015). A new species, *Ptyodactylus ananjevae*, was described from Al Mudawwarah, southern Jordan, very close to Tabuk Province, so it is most likely to also occur in Saudi Arabia (Nazarov et al. 2013).

Family Sphaerodactylidae

The Semaphore geckos of Saudi Arabia are represented by five species. Their taxonomic status was discussed by Arnold (2009). Recently, the status of *Pristurus rupestris* was evaluated on a molecular basis, which showed that *P. rupestris* is restricted to eastern Oman, while a western clade, *Pristurus* sp. 1, is distributed from central coastal Oman, through Yemen, Saudi Arabia, and north to southern Jordan (Badiane et al. 2014). The northwestern population in Saudi Arabia may be assigned as *Pristurus*

guweirensis, however, we still prefer to continue referring to *Pristurus* sp. 1 as *Pristurus rupestris* until further studies validate their separation.

Family Scincidae (Fig. 8)

Ten species of skinks have been reported from Saudi Arabia, and they are represented by six genera (*Ablepharus*, *Chalcides*, *Eumeces*, *Eurylepis*, *Scincus*, and *Trachylepis*). The three species of the genus *Scincus* are strictly sand-dwelling species. *Chalcides levitoni* is known from only one locality in southwestern Saudi Arabia (Pasteur 1978). *Panaspis wahlbergi* was erroneously reported from Saudi Arabia by Al-Jumaily (1984).

Family Lacertidae

Twenty species of lacertids occur in Saudi Arabia. They belong to five genera (*Acanthodactylus*, *Mesalina*, *Ophisops*, *Philochortus*, and *Phoenicolacerta*). Species of the genus *Acanthodactylus* were extensively studied at the molecular level (Tamar et al. 2016a), and constitute the highest number of species, followed by species of the genus *Mesalina*. *Acanthodactylus tilburyi* is known only from Saudi Arabia and southern Jordan (Sindaco and Jeremčenko 2008). Among lizards of the genus *Mesalina*, Sindaco et al. (2018) described *Mesalina arnoldi* from southwestern Saudi Arabia and Yemen, and *Mesalina saudiarabica* was described from Mahazat as-Sayd, near Makkah (Šmíd et al. 2017). By now, *Mesalina brevirostris* is known to be distributed in eastern Saudi Arabia, while *Mesalina bernoullii* is known from northeastern Saudi Arabia (Šmíd et al. 2017). Al-Sadoon et al. (2016) recorded *Acanthodactylus orientalis* and *Acanthodactylus robustus* for the first time from Turaif region. *Phoenicolacerta kulzeri* ssp. was recently recorded from Al Konah, Tabuk (Aloufi and Amr 2015).

Family Trogonophidae (Fig. 9)

This family is represented by a single species in Saudi Arabia. The Zarudny Worm Lizard, *Diplometopon zarudnyi*, was reported from several localities mostly in eastern Saudi Arabia.

Family Varanidae

Two species of the family Varanidae were reported to occur in Saudi Arabia. *Varanus griseus* is widely distributed across Saudi Arabia, while *Varanus yemenensis* is confined to extreme southwestern Saudi Arabia.



Fig. 9. The Zarudnyi Worm Lizard, *Diplometopon zarudnyi*. Photos by A. Aloufi.



Fig. 10. Snakes of Saudi Arabia. (A) *Eryx jayakari*. (B) *Atractaspis engaddensis*. (C) *Echis coloratus*. (D) *Cerastes cerastes*. (E) *Naja arabica*. (F) *Walterinnesia aegyptia*. Photos by A. Al Salman (A–B, F), M. Al Sulimi (C), A. Aloufi (D), and M. Al Mesheni (E).

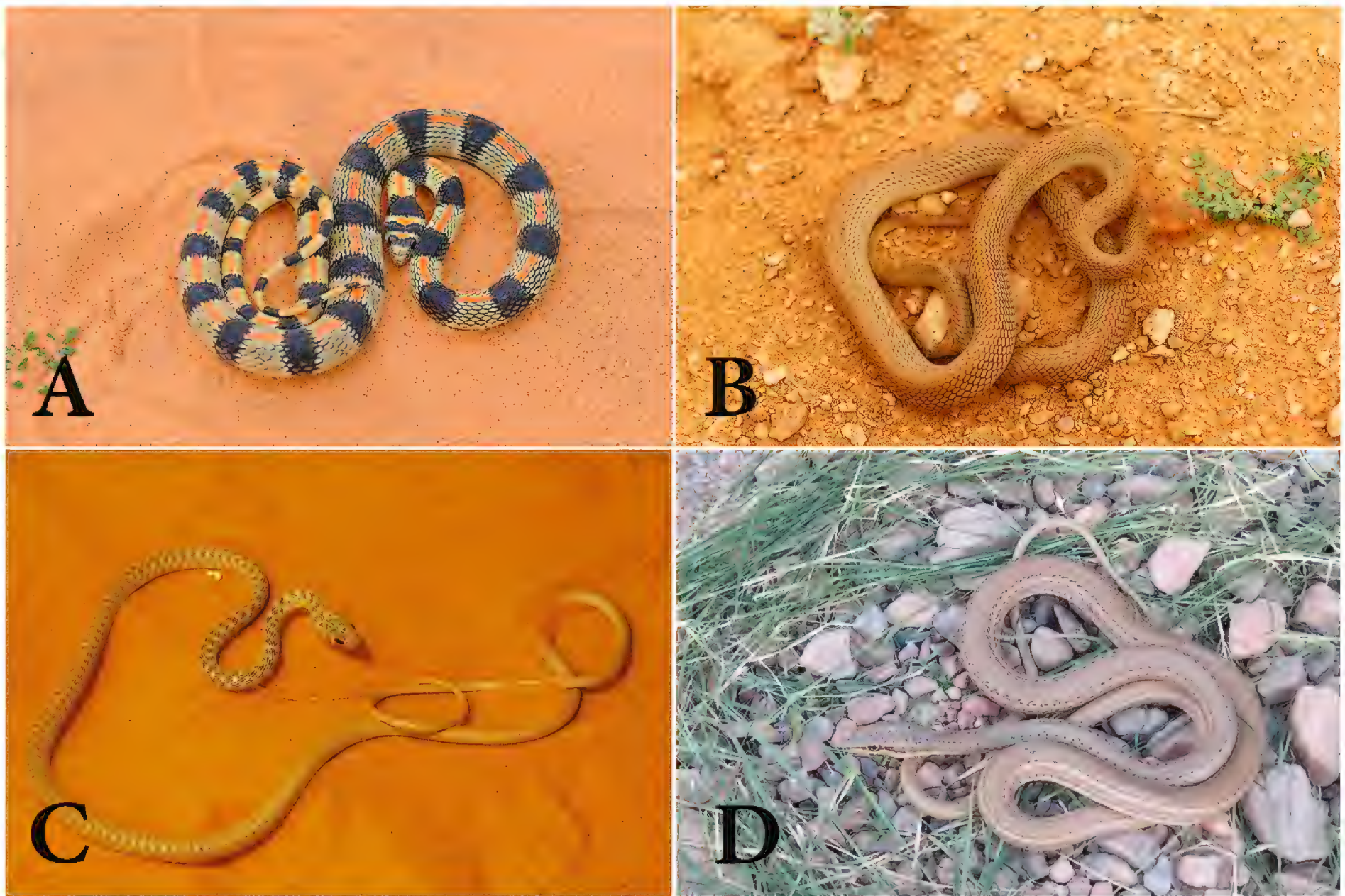


Fig. 11. Snakes of Saudi Arabia. (A) *Platycephalus elagantissimus*. (B) *Telescopus dhara*. (C) *Platycephalus rhodarchis*. (D) *Psammophis schokari*. Photos by M. Al Sulimi (A), A. Al Salman (B–C), and A. Aloufi (D).

Family Leptotyphlopidae

Two species of the genus *Myriopholis*, *Myriopholis macrorhyncha* and *Myriopholis nursii*, have been reported from Saudi Arabia (Egan 2007).

Family Typhlopidae

This family is represented by a single species, *Indotyphlops braminus*. This Asian species is widespread and has become almost cosmopolitan in distribution. It is believed to have been introduced into Arabia through imported plant pots (Egan 2007).

Family Atractaspidae (Fig. 10B)

Mole vipers in Saudi Arabia are exemplified by two species: *Atractaspis andersonii* distributed in southwestern Saudi Arabia, and *Atractaspis engaddensis* in the northwestern and central parts of the country.

Family Boidae (Fig. 10A)

This family is represented by two species. *Eryx jaculus* is known from eastern Saudi Arabia, while *Eryx jayakari* is more common and widespread throughout the country.

Family Colubridae (Fig. 11)

This family includes 13 species and subspecies in six genera (*Dasypeltis*, *Eirenis*, *Lytorhynchus*, *Platycephalus*, *Spalerosophis*, and *Telescopus*). Three species are confined to the extreme southwest of Saudi Arabia (*Platycephalus insulanus*, *Dasypeltis scabra*, and *Lytorhynchus gasperetti*). *Eirenis coronella coronella* is known from eastern Saudi Arabia, while *Eirenis coronella fennelli* is known from the western part of the country. Schätti and McCarthy (2004) confirmed the occurrence of *Platycephalus saharicus* in the Arabian Peninsula. We came across a photographed specimen of *Rhynchocalamus melanocephalus* from Al Konah, near Tabuk and another one from Jabal Al Ward west of Al-'Ula (data not shown). However, the validity of its occurrence in Saudi Arabia requires further specimens.

Family Psammophiidae (Fig. 11D)

This family includes two species in two genera (*Psammophis* and *Rhagerhis*). Both species are desert adapted species with wide ranging distributions across the Kingdom (Gasperetti 1988).

Family Elapidae (Fig. 10E and F)

Three species of terrestrial elapids belonging to two genera (*Naja* and *Walterinnesia*) are known in Saudi

Arabia. Nilson and Rastegar-Pouyani (2007) considered the eastern population of *Walterinnesia* as *Walterinnesia morgani* and the western population as *Walterinnesia aegyptia*. *Naja arabica* is distributed to the west of the country.

Nine species of marine snakes belonging to the genus *Hydrophis* have been reported from the waters of the Arabian Gulf. The nomenclature of sea snakes in Table 1 follow Rezaie-Atagholipour et al. (2016).

Family Viperidae (Fig. 10C and D)

Six species in four genera (*Bitis*, *Cerastes*, *Echis*, and *Pseudocerastes*) of vipers are known in Saudi Arabia. *Bitis arietans*, *Cerastes cerastes*, and *Echis borkini*, are confined to the southwest, while *Pseudocerastes fieldi* occurs to the extreme north on the border with Jordan within Harat Al Harah. *Cerastes gasperetti* is the most common viper, especially in sandy areas.

Relict species

Pelomedusa barbata represents a relict population and is known from southwestern Saudi Arabia (Gasperetti et al. 1993). This African species may have either migrated to southern Arabia or originated in Arabia (Gasperetti et al. 1993; Vargas-Ramírez et al. 2016). The Caspian Turtle, *Mauremys caspica*, reaches the most southeastern range of its distribution around Al Qatif, Al Hufhuf, and Al Uqayr (Gasperetti et al. 1993; Fritz et al. 2008), and these populations are considered as relicts. The population of *Phoenicolacerta kulzeri* ssp. from Al Konah, in the northwest of Saudi Arabia, represents a relict in the sandstone formation of Hisma (Aloufi and Amr 2015).

Endemic species

Seven species are strictly endemic to the Kingdom of Saudi Arabia. *Tropicolotes wolfgangboehmei* is known from one locality in the proximity of Ath-Thumamah, central Saudi Arabia (Wilms et al. 2010). *Chalcides levitoni* was recorded only from Khasawijah, near Jizan (Pasteur 1978). Two snakes, *Platycephalus insulanus*, known only from Sarso Island, Farasan Archipelago (Mertens 1965; Masseti 2014), and *Lytorhynchus gasperetti*, known from two localities in southwestern Saudi Arabia (Leviton 1977), are considered as endemic species. Recently, *Hemidactylus alfarraji* and *Hemidactylus asirensis* were described from southwestern Saudi Arabia (Šmíd et al. 2016), and *Mesalina saudiarabica* was described from Mahazat as-Sayd (Šmíd et al. 2017).

Other species can be considered as endemic at the level of the Arabian Peninsula. Four amphibians (*Dutaphrynus dhufarensis*, *Euphlyctis ehrenbergii*, *Sclerophrys arabica*, and *Sclerophrys tihamica*) are confined to the Arabian Peninsula. *Euphlyctis ehrenbergii* is distributed in southwest Saudi Arabia, and the Riyadh locality

may represent a release or escaped specimens from King Saud University Campus (Al-Johany et al. 2014). Among the reptiles, 20 species are restricted to the Arabian Peninsula excluding Socotra: *Acanthocercus adramitanus*, *Acanthocercus yemenensis*, *Acanthodactylus gongrorhynchatus*, *Acanthodactylus haasi*, *Atractaspis anderssonii*, *Chamaeleo calyptratus calcarifer*, *Echis borkini*, *Mesalina arnoldi*, *Mesalina adramitana*, *Naja arabica*, *Platycephalus variabilis*, *Pristurus carteri*, *Pristurus minimus*, *Pristurus popovi*, *Scincus hemprichii*, *Stenodactylus arabicus*, *Stenodactylus yemenensis*, *Trapelus flavimaculatus*, *Trapelus jayakari*, and *Varanus yemenensis*.

According to the distribution maps given by Sindaco and Jeremčenko (2008), 50 out of 97 lizard species reported from the Arabian Peninsula are considered as endemic to the Peninsula. Twenty-five species of these reptile species are confined to the Arabian Hotspots Areas as outlined by Mallon (2011).

Discussion

The Conservation Status of the Reptiles and Amphibians of Saudi Arabia

Cox et al. (2012) revised the conservation status of the reptiles of the Arabian Peninsula, excluding the marine snakes and turtles. Among them, 101 species are listed as Least Concern, eight are Data Deficient, four are Vulnerable, one is Critically Endangered, and one is Near Threatened (Table 1). However, some species listed as Least Concern, such as *Echis borkini*, *Bitis arietans*, and *Naja arabica*, are under threats due to human practices and their status assessments require revision. Other species that are listed under Data Deficient, such as *Acanthodactylus gongrorhynchatus*, *Chalcides levitoni*, *Lytorhynchus gasperetti*, *Ophisops elbaensis*, *Platycephalus insulanus*, *Tropicolotes wolfgangboehmei*, and *Varanus yemenensis*, have very restricted and very narrow ranges of distribution and so they should be assigned with conservation priorities.

Threats Affecting the Herpetofauna in Saudi Arabia

Four main categories of threats affect the amphibians and reptiles of Saudi Arabia. Some of these threats are very critical for a particular species, while other species may be impacted by more than one type of threat that may lead to population decline.

1. Habitat Loss and Degradation

The human population in Saudi Arabia has increased more than seven-fold in the last 50 years, now reaching up to about 33 million. This rapid growth resulted in the expansion of cities and urban centers at the expense of basic natural resources, especially wildlife and their habitats.

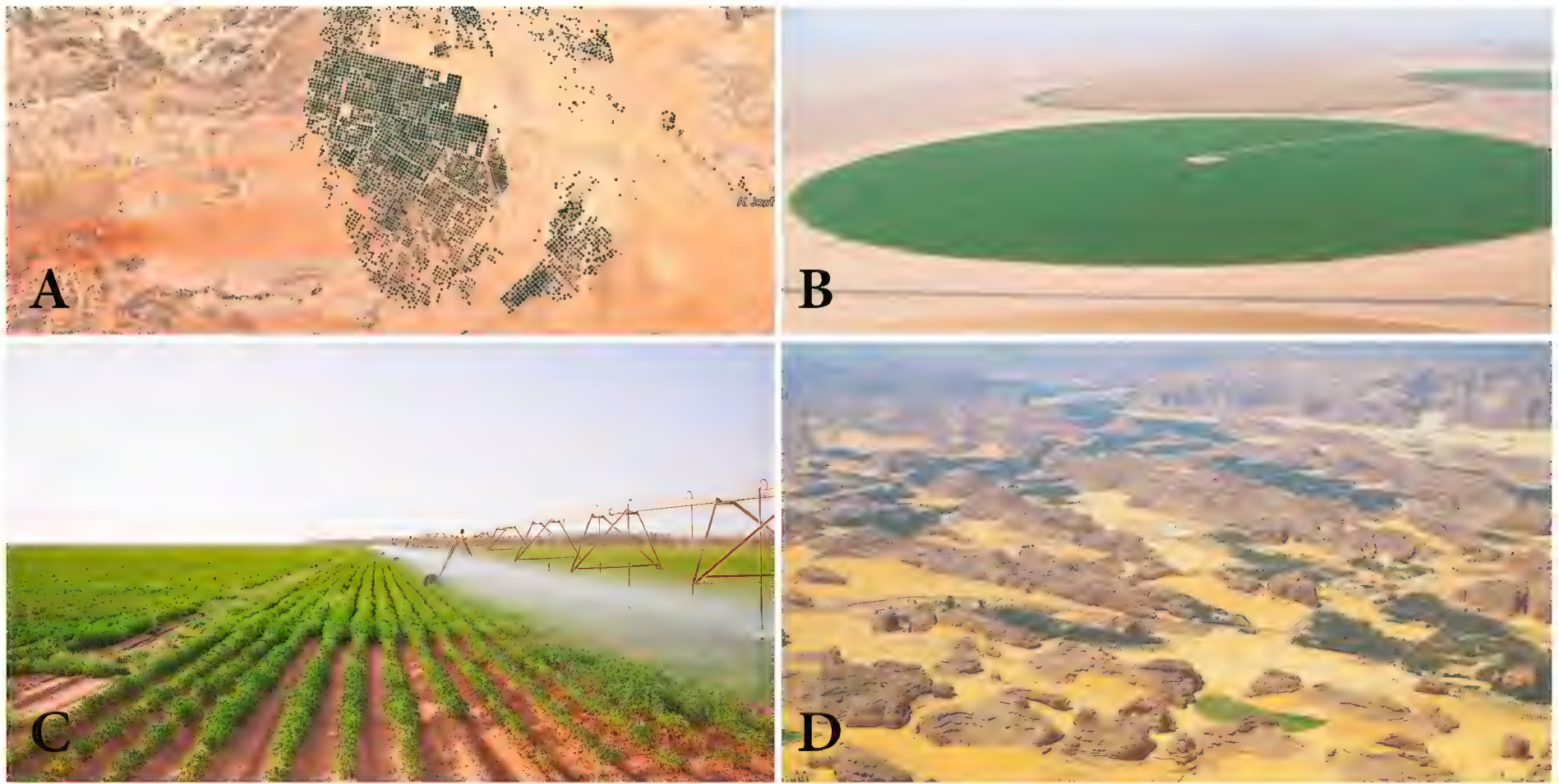


Fig. 12. Habitat disturbance due to farming. **(A)** Fodder farms at Al Jawf. **(B)** Fodder farm in Tabuk. **(C)** Vegetable farms in Tabuk area. **(D)** Farmland in Al-'Ula. Photos by A. Al Rabdi (B–C), and A. Aloufi (D).

Deforestation. It is estimated that forests and woodland cover about 27,000 km² of Saudi Arabia. These woodlands are mostly scattered along the Sarawat mountain in the southwestern region and are dominated by *Juniperus procera*. They have been subjected to misuse through exhaustive wood cutting and overgrazing activities, as well as uncontrolled forest fire and urbanization (El-Juhany 2009). Dieback of *J. procera* forests in the Raydah reserve of southwestern Saudi Arabia was attributed to climate change and the scarcity of rainfall, in addition to extensive farming projects around the area (Fisher 1997).

The southwestern mountains are the home of some rare, endemic, and perhaps endangered species such as *Acanthocercus adramitanus*, *Acanthocercus yemenensis*, *Chalcides levitoni*, *Chamaeleo calypttratus calcarifer*, *Euphlyctis ehrenbergii*, *Lytrohynchus gasperetti*, and *P. barbata*. The conservation status of these species should be assessed in relation to the current state of vegetation cover.

Destruction of the natural vegetation in the desert.

As a result of agricultural expansion in the sand deserts and wadi beds, the clearing of natural vegetation such as *Acacia raddiana*, *Acacia tortilis*, *Moringa peregrine*, *Nitraria retusa*, *Ziziphus spina-christi*, *Retama reaetam*, and *Haloxylon persicum*, and other chenopods has occurred. This clearance had direct effects on the lizards that either use these plants for shading or feed on them. Some species, such as *Trapelus persicus*, take refuge among *Nitraria retusa* shrubs. *Uromastix aegyptia* is an herbivorous lizard that feeds on a variety of desert plants such as *Pennisetum divisum* and *Stipagrostis plumose*, while *Haloxylon salicornicum*, *Polygala erioptera*,

and *Aerva javanica* are consumed to a lesser extent (Cunningham 2000).

Agricultural expansion. Over the past 30 years, large parts of the deserts of Saudi Arabia were dedicated to agricultural projects for the production of wheat, and were converted recently for the production of green fodder and other crops (Fig. 12). Most of these projects are located around Tabuk, Tubarjal, Al Jawf, Hail, Buraydah, Sarj, Al Ula, and Wadi Al Dwasir, and were established since the early 1980s. It is estimated that an area of 694,549 ha was cultivated with fodder, wheat, and other crops in 2013. These projects were established in sandy areas and around wadi courses, due to the availability of groundwater. Alshammari and Ibrahim (2015) found that the smallest numbers of reptiles were collected from cultivated areas in Faid Hema, Ha'il region.

Much destruction of the natural habitats of sand-dwelling species takes place due to the plowing and construction of secondary roads in the desert. The most heavily affected species include *Acanthodactylus schmidtii*, *Cerastes gasperetti*, *Diplometopon zarudnyi*, *Eryx jaculus*, *Lytrohynchus diadema*, *Phrynocephalus nejdensis*, *Scincus hemprichii*, *Scincus mitranus*, *Scincus scincus*, *Stenodactylus doriae*, *Varanus griseus*, and *Uromastix aegyptia*.

Overgrazing. The nomadic lifestyle is still practiced in many parts of Saudi Arabia. Sheep, goats, and camels are among the domestic animals roaming the deserts and the mountains during spring for grazing. This affects reptiles in many ways, including direct disturbance, lowering the vegetation cover, and actually abolishing entire plant communities. Species such as *Trapelus persicus* are af-

fectured by grazing and are displaced from their natural habitats due to the year-round camel grazing on *Nitraria retusa* shrubs.

2. Water Issues

Water extraction and climate change. Intensive farming in many parts of the Saudi deserts for the production of wheat and fodder over the past 30 years has caused drastic changes in the water table. This has led to a dramatic decrease in natural vegetation cover and drying of natural ponds in the desert. The annual rainfall has shown a significant decrease (47.8 mm per decade), with a relatively high inter-annual variability, while temperatures (maximum, mean, and minimum) have increased significantly at rates of 0.71, 0.60, and 0.48 °C, respectively (Almazroui et al. 2012). Eventually, the water re-charge of aquifers will be affected for years to come. According to Alqahtani (2017), *Pelomedusa barbata* is facing threats in southwestern Saudi Arabia as a result of rainfall scarcity in recent years. In Al Hasa region, the number of breeding sites of *Mauremys caspica* was reduced from 159 in the early 1970s to about 19 in 2009, due to destruction of natural springs and construction of cemented canals, along with agricultural expansion (Aloufi 2009).

Pollution and marine debris. Cement dust pollution has affected the Green Turtle, *Chelonia mydas*, in Ras Baridi, reducing hatchling emergence success to only 40% due to the formation of hard domes above the nests which prevent emergence and cause mortality (Pilcher 1999). As a result of urban sewage discharge in open waters of the Red Sea, algal bloom formations have been associated with fibropapillomatosis disease, which is considered deadly to sea turtles (PERSGA/GEF 2004). Marine pollution is also widely believed to affect other sea turtles and snakes.

3. Human Disturbance and Related Activities

Recreational activities and tourism. Camping and driving in the open areas are on the increase in Saudi Arabia. Large vehicles and desert dirt bikes are widely used for racing on the sand dunes. This will certainly affect many sand-dwelling lizard and snake species, and cause disturbance (Table 1). At the same time, many shrubs and plants are destroyed during vehicle movement across the terrain. Tourism causes disturbance to fragile sensitive ecosystems, especially in places where relict or endangered species exist.

Direct persecution. Reptiles in general, and snakes in particular, are widely disliked animals. Many photos can be found posted on social media which show snakes that have been killed. Among them are venomous species, *Atractaspis andersonii*, *Atractaspis engaddensis*, *Cerastes gasperetti*, *Echis coloratus*, *Naja arabica*, and

Walterinnesia aegyptia. However, non-venomous snakes, such as *Spalerosophis diadema cliffordii*, *Psammophis schokari*, and *Platycephalus rhodarchis*, are also often killed instantly even when they are encountered in the wilderness. In Saudi Arabia, all forms of geckos are killed since it is widely believed that they transmit leprosy.

Trade and commercial collection. We observed five species of snakes that were traded in the animal markets in Jeddah, Tabuk, Taif, and Al Madinah Al Monawarh: *Eryx jaculus*, *Naja arabica*, *Psammophis schokari*, *Platycephalus rhodarchis*, and *Spalerosophis diadema cliffordii*. These snakes were cramped in plastic bottles and directly exposed to the sun. Lizards that were traded in the animal markets included *Chamaeleo chamaeleon*, *Chamaeleo calypttratus*, *Scincus mitranus*, *Scincus scincus*, *Uromastix aegyptia*, *Uromastix ornatus*, *Varanus yemenensis*, and *Varanus griseus*.

The Hawksbill Turtle, *Eretmochelys imbricata*, is collected from the Red Sea and sold in animal markets in Tabuk (Aloufi and Eid 2014). A less common item of trade is the Western Caspian Turtle, *Mauremys caspica*, but it can also be found in animal markets and pet shops (Aloufi and Eid 2014) and sold in Riyadh and Al Hasa Province. The population density of *Pelomedusa barbata* is under stress and has decreased greatly over the last few years in southwestern Saudi Arabia. However, it is commonly taken from its freshwater habitats in the southwest for trade (Alqahtani 2017).

The Spiny-tailed Lizard is sold for its meat, while skinks (*Scincus mitranus* and *Scincus scincus*) are sold as dried preparations that are prescribed in folk medicine as aphrodisiacs. For animal exhibits and shows, snakes such as *Eryx jaculus*, *Naja arabica*, and *Psammophis schokari* are in demand. Most of these animals are simply disposed of after the shows, and it has been estimated that over 100 Arabian cobras were killed in one season. For teaching purposes, university students and high schools purchase various amphibians, desert monitors, and spiny-tailed lizards.

Hunting and pouching. The consumption of eggs and meat of marine turtles has been reported in Saudi Arabia (Miller 1989), however, this practice is not very common. However, the major problem of hunting and direct harvesting of the Egyptian Spiny-tailed Lizard, *Uromastix aegyptia*, is alarming. Thousands of these animals are captured for human consumption and trade. The locals relish both the meat and the eggs. Truckloads of dead and slaughtered *dabbs* are posted on the hunter internet sites as a sign of pride (Fig. 13). Gravid females are in high demand due to their eggs. We counted over 17 females killed in one hunting trip, and females typically lay 17–41 eggs in one clutch during May or June (Bouskila 1984). This excessive harvest will certainly affect the population of *U. aegyptia*, whereas large numbers of females are killed in the eggs that are not



Fig. 13. The Egyptian Spiny-tailed Lizard, *Uromastix aegyptia*, is hunted and killed by the hundreds and sold either alive or for its meat and eggs.

allowed to hatch. Both vitellogenic and oviductal eggs were observed among the females that had been killed (Fig. 13).

4. Legislative and Public Awareness

Enforcement. Although all wild animals in Saudi Arabia are protected by law, enforcement is still far behind in protecting the animals, and particularly reptiles. In fact, the Spiny-tailed Lizard, *U. aegyptia*, is included among the animals that are allowed to be hunted, together with birds. The hunting season for the Spiny-tailed Lizard is open from the beginning of August to the end of September outside of the protected areas, with no bag limit. This ambiguity in the number of allowed animals has led to a massive scale of hunting and killing of this vulnerable species. Until recently, the concept of conserving diversity has remained obscure to many decision makers in Saudi Arabia. The broad spectrum of biological diversity in the country requires trained individuals to reveal its importance for the country with

respect to various aspects, including socio-economic, ecotourism, scenic, and ethical perspectives.

Public awareness. As stated earlier, reptiles are disliked by most of the locals, and surrounded by mystery and superstitions. The general public attitude towards reptiles is a mix of aversion and fear that stems from deep-rooted traditional culture. Therefore, public awareness remains one of the most important issues for introducing the importance of reptiles to the general public; and public awareness of the importance of conservation is a high priority issue. The role of non-governmental organizations is to introduce wildlife conservation to various sectors of the community, mainly children and youngsters, as well as to rally support from decision makers.

The environmental public awareness towards animals in the Kingdom of Saudi Arabia remains limited, and is almost totally lacking for reptiles. The availability of books for providing scientific information to the public or at the level of high school education remains very limited. The lack of understanding in the general public is clearly illustrated by social media being so full of videos

and images of animal persecution and overhunting; with expression of great pride in such actions.

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Literature Cited

- Al-Johany AM. 1995. The ecology of *Agama yemenesis* Klausewitz (Lacertilia: Agamidae) in south-western Arabia. *Journal of Arid Environments* 29: 495–503.
- Al-Johany AM, Al-Qarni SS, Hasayen KA. 2014. Distribution and habitats of amphibians in the central region of Saudi Arabia. *Herpetological Conservation and Biology* 9: 601–608.
- Al-Jumaily MM. 1984. Reptiles of Saudi Arabia. *Panaspis wahlbergi* (Smith, 1849), an addition to the reptile fauna of Saudi Arabia. *Fauna of Saudi Arabia* 6: 528–553.
- Almazroui M, Nazrul Islam M, Athar H, Jones PD. 2012. Recent climate change in the Arabian Peninsula: annual rainfall and temperature analysis of Saudi Arabia for 1978–2009. *International Journal of Climatology* 32: 953–966.
- Al-Merghani M, Miller JD, Pilcher NJ, Al-Mansi A. 2000. The green and hawksbill turtles in the Kingdom of Saudi Arabia: synopsis of nesting studies 1986–1997. *Fauna of Arabia* 18: 369–384.
- Al-Nafie AH. 2008. Phytogeography of Saudi Arabia. *Saudi Journal of Biological Sciences* 15: 159–176.
- Al-Nafie AH. 2018. *Zoogeography of Wild Animals in Saudi Arabia*. Riyadh, Kingdom of Saudi Arabia. 23 p.
- Aloufi AA. 2009. Ecological aspects of freshwater turtle *Mauremys caspica siebenrocki*, in Al Hasa Region, Saudi Arabia. Ph.D. Thesis. King Saud University, Riyadh, Saudi Arabia. 288 p.
- Aloufi A, Eid E. 2014. Conservation perspectives of illegal animal trade at Tabuk local market, Kingdom of Saudi Arabia. *Traffic Bulletin* 26: 77–80.
- Aloufi AA, Amr ZS. 2015. On the herpetofauna of the Province of Tabuk, northwest Saudi Arabia (Amphibia, Reptilia). *Herpetozoa* 27: 147–158.
- Al-Qahtani AR, Al-Johany AW. 2018. Amphibians distribution and habitats in the southwestern region of Saudi Arabia. *Saudi Journal of Biological Sciences* 25: 1,380–1,386.
- Alqahtani ARM. 2017. Revision of geographical distribution of *Pelomedusa subrufa* in Saudi Arabia. *King Khalid University Journal of Basic & Applied Sciences* 3: 26–28.
- Alqahtani ARM. 2018. Lizard community in Tathleeth District of southwestern Saudi Arabia. *Egyptian Academic Journal of Biological Sciences* 10: 29–34.
- Al-Sadoon MK. 1988. Survey of the reptilian fauna of the Kingdom of Saudi Arabia. II. The lizard and amphisbaenian fauna of Riyadh Province. *Bulletin of the Maryland Herpetological Society* 24: 58–76.
- Al-Sadoon MK. 1989. Survey of the reptilian fauna of the kingdom of Saudi Arabia I – the snake fauna of the central region. *Journal of King Saud University - Science* 1: 53–69.
- Al-Sadoon MK. 2010. Survey of the reptilian fauna of the kingdom of Saudi Arabia IV. The lizards, snakes and amphisbaenian fauna of Al-Hassa Region. *Journal of the Egyptian-German Society of Zoology. Series B* 61: 59–85.
- Al-Sadoon MK, Al-Farraj SA, Abdo NM. 1991. Survey of the reptilian fauna of the kingdom of Saudi Arabia. III. An ecological survey of the lizard, amphisbaenian and snake fauna of Al-Zulfi Area. *Bulletin of the Maryland Herpetological Society* 27: 1–22.
- Al-Sadoon MK, Paray BA, Al-Otaibi HS. 2016. Survey of the reptilian fauna of the Kingdom of Saudi Arabia. V. The lizard fauna of Turaif region. *Saudi Journal of Biological Sciences* 23: 642–648.
- Al-Shammari MA. 2012. Additional records of lizards in Ha'il Province, Saudi Arabia. *Russian Journal of Herpetology* 19: 287–291.
- Alshammari AH, Busais SM, Ibrahim AA. 2017. Snakes in the Province of Ha'il, Kingdom of Saudi Arabia, including two new records. *Herpetozoa* 30: 59–63.
- Alshammari AM, Ibrahim AA. 2015. Lizards and snakes in the historical Faid Protected Area (Faid Hema), Ha'il region, Saudi Arabia. *Herpetological Conservation and Biology* 10: 1,021–1,029.
- Alshammari A, Ibrahim AA. 2018. Amphibians of the Ha'il region, Saudi Arabia, with special reference to their habitat and distribution. *Advances in BioResearch* 9: 165–172.
- Al-Wailly AJ, Al-Uthman HS. 1971. Some lizards from Central Saudi Arabia. *Bulletin of the Iraq Natural History Museum* 5: 39–42.
- Ananjeva NB, Patrick D, Barabanov A, Dubois A. 2013. On the type specimens of *Trapelus ruderatus* (Olivier, 1804) and some nomenclatural problems on *Trapelus* Cuvier, 1816 (Agamidae, Sauria). *Russian Journal of Herpetology* 20: 197–202.
- Arnold EN. 1980. Reptiles of Saudi Arabia. A review of the lizard genus *Stenodactylus* (Reptilia: Gekkonidae). *Fauna of Saudi Arabia* 2: 368–404.
- Arnold EN. 2009. Relationships, evolution, and biogeography of Semaphore geckos, *Pristurus* (Squamata, Sphaerodactylidae) based on morphology. *Zootaxa* 2060: 1–21.
- Arnold EN. 1986. A key and annotated checklist to the lizards and amphisbaenians of Arabia. *Fauna of Saudi*

- Arabia* 8: 385–435.
- Badiane A, Garcia-Porta J, Červenka J, Kratochvíl L, Sindaco R, Robinson MD, Morales H, Mazuch T, Price T, Amat F, et al. 2014. Phylogenetic relationships of Semaphore geckos (Squamata: Sphaerodactylidae: *Pristurus*) with an assessment of the taxonomy of *Pristurus rupestris*. *Zootaxa* 3835: 33–58.
- Balletto EM, Cherchi MA, Gasperetti J. 1985. Amphibians of the Arabian Peninsula. *Fauna of Saudi Arabia* 7: 318–392.
- Bouskila A. 1984. Habitat selection, in particular burrow location, in the dabb-lizard *Uromastix aegyptius*, near Hazeva. M.Sc. Thesis, Hebrew University of Jerusalem, Jerusalem, Israel [in Hebrew].
- Carranza S, Xipell M, Tarroso P, Gardner A, Arnold EN, Robinson MD, Simó-Riudalbas M, Vasconcelos R, de Pous P, Amat F, et al. 2018. Diversity, distribution, and conservation of the terrestrial reptiles of Oman (Sauropsida, Squamata). *PLoS ONE* 13(2): e0190389.
- Cogălniceanu D, Castilla AM, Valdeón A, Gosá A, Al-Jaidah N, Alkuwary A, Saifelnasr EOH, Mas-Peinado P, Richer R, Al-Hemaidi AAM. 2014. A preliminary report on the distribution of lizards in Qatar. *ZooKeys* 373: 67–91.
- Cox NA, Mallon D, Bowles P, Els J, Tognelli MF. (compilers) (2012). *The Conservation Status and Distribution of Reptiles of the Arabian Peninsula*. IUCN, Cambridge, United Kingdom and Gland, Switzerland, and Environment and Protected Areas Authority, Sharjah, United Arab Emirates. 39 p.
- Cunningham P. 2000. Daily activity pattern and diet of a population of the Spiny-tailed Lizard, *Uromastix aegyptius microlepis*, during summer in the United Arab Emirates. *Zoology in the Middle East* 21: 37–46.
- Cunningham PL. 2010. Checklist of terrestrial reptiles in three protected areas in the Kingdom of Saudi Arabia. *Herpetological Review* 41: 25–28.
- Dekinesh SI. 1991. The reptilian fauna of Hail district, north of Saudi Arabia. *Journal of the Egyptian-German Society of Zoology* 5: 177–196.
- Disi A, Amr ZS, Hamidan N. 2014. Diversity, threats, and conservation of the terrestrial and freshwater herpetofauna of Jordan. *Russian Journal of Herpetology* 21: 221–233.
- Egan D. 2007. *Snakes of Arabia*. Motivate Publishing, Dubai, United Arab Emirates. 208 p.
- El-Juhany LI. 2009. Forestland degradation and potential rehabilitation in southwest Saudi Arabia. *Australian Journal of Basic and Applied Sciences* 3: 2,677–2,696.
- Farag AA, Banaja AA. 1980. Amphibians and reptiles from the western region of Saudi Arabia. *Bulletin of Science of King Abdul Aziz University* 4: 5–29.
- Fisher M. 1997. Decline in the juniper woodlands of Raydah reserve in southwestern Saudi Arabia: a response to climate changes? *Global Ecology and Biogeography Letters* 6: 379–386.
- Fritz U, Ayaz D, Buschbom J, Kami HG, Mazanaeva LF, Aloufi AA, Auer M, Rifai L, Šilić T, Hundsdoerfer AK. 2008. Go east: phylogeographies of *Mauremys caspica* and *M. rivulata* – discordance of morphology, mitochondrial and nuclear genomic markers, and rare hybridization. *Journal of Evolutionary Biology* 21: 527–540.
- Gardner AS. 2013. *The Amphibians and Reptiles of Oman and the UAE*. Chimaira, Frankfurt am Main, Germany. 480 p.
- Gasperetti J, Stimson FA, Miller JD, Ross JP, Gasperetti PR. 1993. Turtles of Arabia. *Fauna of Saudi Arabia* 13: 170–367.
- Gasperetti J. 1988. Snakes of Arabia. *Fauna of Saudi Arabia* 9: 169–450.
- Gvožik V, Moravec J, Klütsch C, Kotik P. 2010. Phylogeography of the Middle Eastern tree frogs (*Hyla*, Hylidae, Amphibia) as inferred from nuclear and mitochondrial DNA variation, with a description of a new species. *Molecular Phylogenetics and Evolution* 55: 1,146–1,166.
- Haas G. 1957. Some amphibians and reptiles from Arabia. *Proceedings of the California Academy of Sciences* 29: 47–86.
- Haas G, Werner YL. 1969. Lizards and snakes from Southwestern Asia, collected by Henry Field. *Bulletin of the Museum of Comparative Zoology* 138: 327–406.
- Hillenius D, Gasperetti J. 1984. Reptiles of Saudi Arabia: the chameleons of Saudi Arabia. *Fauna of Saudi Arabia* 6: 513–527.
- Hussein HK, Darwish AD. 2001. A survey of the herpetofauna of Bisha district, south of Saudi Arabia. *Journal of Biological Sciences* 1: 728–730.
- Leviton AE. 1977. A new lytorhynchid snake. *Journal of the Saudi Arabian Natural History Society* 19: 16–25.
- Leviton AE, Anderson SC, Adler K, Minton SA. 1992. *Handbook to Middle East Amphibians and Reptiles*. Contributions to Herpetology, Number 8. Society for the Study of Amphibians and Reptiles, Oxford, Ohio, USA. 252 p.
- Mallon DP. 2011. Global hotspots in the Arabian Peninsula. *Zoology in the Middle East* (Supplementum 3): 13–20.
- Mancini A, Elsadek I, Elawani M. 2015. Marine turtles of the Red Sea. Pp. 551–565 In: *The Red Sea*. Editors, Rasul N, Stewart ICF. Springer-Verlag, Berlin, Germany. 638 p.
- Masood MF. 2012. Ecological distribution of snakes' fauna of Jazan region of Saudi Arabia. *Egyptian Academy Journal of Biological Sciences* 4: 183–197.
- Masood MF, Asiry AA. 2012. Ecological studies on diversity of herpetofauna in Asir region, Kingdom of Saudi Arabia. *Egyptian Academy Journal of Biological Sciences* 4: 143–163.
- Masetti M. 2014. Herpetological enigmas from the Arabian seas, with particular reference to the Sarso island racer, *Platyceps insularis* Mertens, 1965 (Farasan archipelago, Saudi Arabia). Pp. 99–116 In:

- Studies on Amphibians and Reptiles in Honour of Benedetto Lanza*. Scripta Herpetologica. Edizioni Belvedere, Latina, Italy. 200 p.
- Melnikov D, Melnikova E, Nazarov R, Rajabizadeh M, Al-Johany A, Amr ZS, Ananjeva NB. 2014. Taxonomic revision of *Phrynocephalus arabicus* Anderson, 1984 complex with description of a new species from Ahvaz, South-Western Iran. *Russian Journal of Herpetology* 21: 149–159.
- Mertens R. 1965. Eine neue Natter von einer Insel des roten Meeres. *Senckenbergiana Biologica* 46: 5–9.
- Metallinou M, Arnold EN, Crochet P-A, Geniez P, Brito JC, Lymberakis P, Baha El Din S, Sindaco R, Robinson MD, Carranza S. 2012. Conquering the Sahara and Arabian deserts: systematics and biogeography of *Stenodactylus* geckos (Reptilia: Gekkonidae). *BMC Evolutionary Biology* 12: 1–17.
- Metallinou M, Červenka J, Crochet P-A, Kratochvíl L, Wilms T, Geniez P, Shobrak MY, Brito JC, Carranza S. 2015. Species on the rocks: Systematics and biogeography of the rock-dwelling *Ptyodactylus* geckos (Squamata: Phyllodactylidae) in North Africa and Arabia. *Molecular Phylogenetics and Evolution* 85: 208–220.
- Nazarov R, Melnikov D, Melnikova K. 2013. Three new species of *Ptyodactylus* (Reptilia; Squamata; Phyllodactylidae) from the Middle East. *Russian Journal of Herpetology* 20: 147–162.
- Nazarov, RA, Melnikov DA, Radjabizadeh M, Poyarkov NA. 2018. A new species of short-fingered geckos *Stenodactylus* (Squamata, Gekkonidae) from South Iran with taxonomic notes on validity of the genus *Trigonodactylus* Hass, 1957. *Zootaxa* 4457(1): 93–113.
- Nilson G, Rastegar-Pouyani N. 2007. *Walterinnesia aegyptia* Lataste, 1887 (Ophidia: Elapidae) and the status of *Naja morgani* Mocquard 1905. *Russian Journal of Herpetology* 14: 7–14.
- Pasteur J. 1978. Note sur les sauriens du genre *Chalcides*. III. Description de *Chalcides levitoni* n. sp. D'Arabie Saoudite (Reptilia, Lacertilia, Scincidae). *Journal of Herpetology* 12: 371–372.
- PERSGA/GEF. 2004. *Regional Action Plan for the Conservation of Marine Turtles and their Habitats in the Red Sea and Gulf of Aden*. PERSGA, Jeddah, Saudi Arabia. 65 p.
- Petzold A, Vargas-Ramírez M, Kehlmaier C, Vamberger M, Branch WR, Du Preez L, Hofmeyr MD, Meyer L, Schleicher A, Siroký P, et al. 2014. A revision of African helmeted terrapins (Testudines: Pelomedusidae: *Pelomedusa*), with descriptions of six new species. *Zootaxa* 3795(5): 523–548.
- Pilcher NJ. 1999. Cement dust as a cause of sea turtle hatchling mortality at Ras Baridi, Saudi Arabia. *Marine Pollution Bulletin* 38: 966–969.
- Pilcher NJ, Antonopoulou B, Perry L, Abdel-Moati MA, Al Abdessalaam TZ, Albeldawi M, Al Ansi M, Al-Mohannadi SF, Al Zahlawi N, Baldwin R, et al. 2014. Identification of Important Sea Turtle Areas (ITAs) for hawksbill turtles in the Arabian Region. *Journal of Experimental Marine Biology and Ecology* 460: 89–99.
- Rastegar-Pouyani N. 2000. Taxonomic status of *Trapelus ruderatus* (Olivier) and *T. persicus* (Blanford), and validity of *T. lessonae* (De Filippi). *Amphibia-Reptilia* 21: 91–102.
- Rezaie-Atagholipour M, Ghezellou P, Hesni MA, Dakhteh SMH, Ahmadian H, Vidal N. 2016. Sea snakes (Elapidae, Hydrophiinae) in their westernmost extent: an updated and illustrated checklist and key to the species in the Persian Gulf and Gulf of Oman. *ZooKeys* 622: 129–164.
- Ross JP. 1985. Identification of marine turtles in the Red Sea. *Journal of Saudi Arabia Natural History Society* 2: 12–21.
- Schleich HH, Kästle W, Kabisch K. 1996. *Amphibians and Reptiles of North Africa*. Koeltz, Koenigstein, Germany. 627 p.
- Schätti B, Gasperetti J. 1994. A contribution to the herpetofauna of Southwest Arabia. *Fauna of Saudi Arabia* 14: 384–423.
- Schätti B, McCarthy C. 2004. Saharo-Arabian racers of the *Platycephalus rhodorhachis* complex - description of a new species (Reptilia: Squamata: Colubrinae). *Revue Suisse de Zoologie* 111(4): 691–705.
- Schmidt KP. 1941. Reptiles and Amphibians in central Arabia. *Field Museum of Natural History (Series Zoology)* 24: 161–165.
- Sindaco R, Jeremčenko VK. 2008. *The Reptiles of the Western Palearctic. Volume 1. Annotated Checklist and Distributional Atlas of the Turtles, Crocodiles, Amphisbaenians and Lizards of Europe, North Africa, Middle East and Central Asia*. Edizioni Belvedere, Latina, Italy. 579 p.
- Sindaco R, Venchi A, Grieco C. 2013. *The Reptiles of the Western Palearctic, Volume 2: Annotated Checklist and Distributional Atlas of the Snakes of Europe, North Africa, Middle East and Central Asia, with an Update to Volume 1*. Edizioni Belvedere, Latina, Italy. 543 p.
- Sindaco R, Nincheri R, Lanza B. 2014. Catalogue of Arabian reptiles in the collections of the “La Specola” Museum, Florence. Pp. 137–164 In: *Studies on Amphibians and Reptiles in Honour of Benedetto Lanza*. Scripta Herpetologica. Edizioni Belvedere, Latina, Italy. 200 p.
- Sindaco R, Simó-Riudalbas M, Sacchi R, Carranza S. 2018. Systematics of the *Mesalina guttulata* species complex (Squamata: Lacertidae) from Arabia with the description of two new species. *Zootaxa* 4429: 513–547.
- Šmíd J, Moravec J, Kratochvíl L, Gvoždík V, Nasher AK, Busais SM, Wilms T, Shobrak MY, Carranza S. 2013. Two newly recognized species of *Hemidactylus*

- (Squamata, Gekkonidae) from the Arabian Peninsula and Sinai, Egypt. *ZooKeys* 355: 79–107.
- Šmíd J, Shobrak M, Wilms T, Joger U, Carranza S. 2016. Endemic diversification in the mountains: genetic, morphological, and geographical differentiation of the *Hemidactylus* geckos in southwestern Arabia. *Organisms Diversity & Evolution* 17: 267–285.
- Šmíd J, Moravec J, Gvoždík V, Štundl J, Frynta D, Lymberakis P, Kapli P, Wilms T, Schmitz A, Shobrak M, et al. 2017. Cutting the Gordian Knot: phylogenetic and ecological diversification of the *Mesalina brevirostris* species complex (Squamata, Lacertidae). *Zoologica Scripta* 46: 649–664.
- Tamar K, Carranza S, Sindaco R, Moravec J, Trape JF, Meiri S. 2016a. Out of Africa: phylogeny and biogeography of the widespread genus *Acanthodactylus* (Reptilia: Lacertidae). *Molecular Phylogenetics and Evolution* 103: 6–18.
- Tamar K, Scholz S, Crochet P-A, Geniez P, Meiri S, Schmitz A, Wilms T, Carranza S. 2016b. Evolution around the Red Sea: systematics and biogeography of the agamid genus *Pseudotrapelus* (Squamata: Agamidae) from North Africa and Arabia. *Molecular Phylogenetics and Evolution* 97: 55–68.
- Uetz P, Freed P, Hošek J. (Editors). 2018. The Reptile Database. Available: <http://www.reptile-database.org> [Accessed: 1 December 2018].
- Vamberger M, Stuckas H, Ayaz D, Graciá E, Aloufi AA, Els J, Mazanaeva LF, Kami HG, Fritz U. 2013. Conservation genetics and phylogeography of the poorly known Middle Eastern terrapin *Mauremys caspica* (Testudines: Geoemydidae). *Organisms Diversity & Evolution* 13: 77–85.
- Vargas-Ramírez M, Petzold A, Fritz U. 2016. Distribution modelling and conservation assessment for helmeted terrapins (*Pelomedusa* spp.). *Salamandra* 52: 306–316.
- Wilms TM, Böhme W. 2007. Review of the taxonomy of the spiny-tailed lizards of Arabia (Reptilia: Agamidae: Leiolepidinae: *Uromastyx*). *Fauna of Arabia* 23: 435–468.
- Wilms TM, Shobrak M, Wagner P. 2010. A new species of the genus *Tropicolotes* from Central Saudi Arabia (Reptilia: Sauria: Gekkonidae). *Bonn Zoological Bulletin* 57: 27–280.



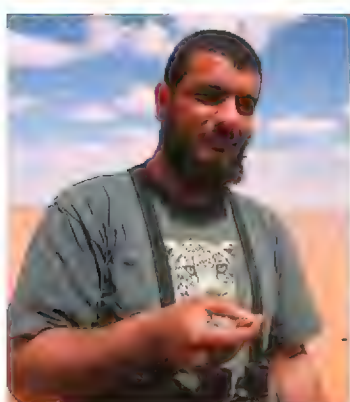
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Phylogenetic analysis of the Common Krait (*Bungarus caeruleus*) in Pakistan based on mitochondrial and nuclear protein coding genes

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Abstract.—Pakistan has more than 40 species of venomous snakes. One of them, the Common Krait (*Bungarus caeruleus*), is responsible for most of the reported snake bites followed by Russel's Viper, Saw-scaled Viper, and Black Cobra. Molecular studies not only help in correctly identifying organisms but also in finding the phylogenetic relationships and diversity among and between them. Morphological studies can be supplemented with confirmatory molecular data to make them more authentic and accurate. This study is the first to characterize the genetic diversity and phylogenetic relationships of Common Kraits from Pakistan, which will help in developing effective strategies for managing snake bites through effective antivenom development. Tail tip biopsies of 25 Common Kraits were collected from different cities in Pakistan. The whole DNA was extracted. Four mitochondrial (ND4, Cytochrome b, 12S rRNA, and 16S rRNA) and three nuclear protein coding (C-mos, RAG-1, and NT3) gene fragments were amplified using specific PCR primers. The amplified DNA was sequenced by Sanger di-deoxy sequencing. Forward and reverse sequences were cleaned and contiged using Sequencher 5.0 software. DNA data were aligned and concatenated using MEGA 6.0 and SequenceMatrix software, respectively. Partition Finder software was used for obtaining the best partitioning scheme and evolutionary models. Concatenated maximum likelihood and Bayesian phylogenetic trees were constructed using RaxML and MrBayes software. The same alignments were used to perform DNA polymorphism analysis using DnaSP 5.0 software. A percent identity matrix was created for all sequences using the online bioinformatics tool, MUSCLE. Homology was presented in tabular form, showing the similarity among different species of genus *Bungarus*. All *Bungarus* species were differentiated into four groups. Common Krait (*B. caeruleus*) from Pakistan showed close relationships with *B. sindanus* and *B. ceylonicus*, as one monophyletic group. The first clade included *B. candidus* (Indonesia, Thailand, Vietnam, and Laos), *B. multicinctus* (China, Taiwan, and Burma), and *B. niger* (Nepal). The second clade included *B. sindanus* and *B. caeruleus* (Pakistan), and *B. ceylonicus* (Sri Lanka). The third clade included *B. fasciatus* (Thailand and Indonesia), while the fourth clade included *B. bungroides* (China) and *B. flaviceps* (Malaysia and Indonesia). This study traces the diversity and phylogenetic relationships of the Pakistani elapid, Common Krait, showing the considerable inter- and intra-specific variations from different geographical regions of the world.

Keywords. Asia, Elapidae, PCR, polymorphism, Serpentes, venomous snakes

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Introduction

Snakes are legless carnivorous reptiles of suborder Serpentes, and their lack of eyelids and external ears distinguish them from legless lizards (Reeder et al. 2015). Except Antarctica and some other large islands, like Ireland, Iceland, Greenland, the Hawaiian archipelago,

and New Zealand islands, snakes are found everywhere in the world (Roland 1994). South Asia is the region most affected with venomous snake bites. For example, the World Health Organization reports 35,000 to 50,000 deaths annually in India (Chippaux 1998; Pyron et al. 2013), and Pakistan reports 40,000 snake bites every year that result in 8,200 fatalities (Pyron et al. 2013).

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Fig. 1. Sample collection sites in Pakistan for Common Krait (*Bungarus caeruleus*).

Venomous snakes in Southeast Asia belong to families Elapidae (cobras and kraits) and Viperidae (typical vipers and pit vipers). A study of hospital-admitted snakebite cases in Pakistan revealed less than 5% neurotoxic snakebites, and the rest were viper bites (Nisar et al. 2009).

Kraits, genus *Bungarus*, are identified by alternating black and white cross-bands across the body, and are found in all South Asian countries except the Philippines. Members of genus *Bungarus* are moderate to large sized elapids distributed in Pakistan and eastward through southern Asia to Indonesia (Smith 1943). Currently, 12 species of kraits are recognized (Yulin et al. 2018), including three in Pakistan. In Pakistan, Common Kraits (*Bungarus caeruleus*) are reported throughout Punjab, Khyber Pakhtoonkhwa (KPK), Azad Kashmir, Sindh, and Southern Balochistan. Common in the Indus valley, this is the only species of kraits found in Rawalpindi and Islamabad (Khan 2002a; Oh et al. 2019). Sindhi Krait (*B. sindanus*) is prevalent in Tharparkar, Bahawalnagar, and Bahawalpur. Northern Punjab Krait (*B. razai*) is reported from Mianwali (Khan 2002b). Determining the relationships among the members of Elapidae can help in understanding their distribution and diversity. Many studies have focused on the evolutionary relationships of kraits.

Before the widespread use of DNA sequencing, systematics and taxonomy were used to infer phylogenies among species in order to explain their relationships. Now many fields in biology are using phylogenies for a wide variety of purposes, such as examining paralogous relationships, population histories, dynamics of pathogens with respect to their evolution and epidemiology (Zhou et al. 2018; Blanquart 2019), the ontogeny of body cells during development, and the differentiation of tumors (Kester and van Oudenaarden 2018). Variations in nucleotide sequences can construct phylogenies for

inferring relationships among the compared sequences. The topology of phylogeny gives some estimates about the mutation rates, time-scales of evolutionary events, and prehistoric movement among different geographical regions (Soroka and Burzyński 2018). Phylogenetics shows relationships among organisms and genes (Friberg et al. 2019), and can give a clearer picture of the biodiversity, biogeography, and evolution of many characters in related groups (Pilfold et al. 2019; Grismer and Davis 2018; Silva et al. 2019).

The use of mitochondrial DNA data for studying animal evolution has become a powerful tool in the last decade. Molecular biology has helped in these mitochondrial DNA studies to give insights into population structure, gene flow, hybridization, biogeography, and phylogenetics (Chandrasekaran et al. 2019; Soroka and Burzyński 2018). Evolutionary studies give comparisons of mitochondrial genome organization and function while molecular studies help to improve these evolutionary studies (Ng et al. 2019). Nuclear encoded genes seem to be a strong source of phylogenetic information. They can be more useful for showing the divergence of those genes whose multiple substitutions may obscure clear phylogenetic signals.

This is the first study from Pakistan focusing on genetic characterization, biodiversity, and molecular phylogenetics of the Common Krait (*Bungarus caeruleus*).

Materials and Methods

A collection of 25 Common Kraits (*Bungarus caeruleus*) was obtained from reptile breeders in different cities in Pakistan (see Fig. 1, Table 1). Scalation patterns and

Table 1. Common Krait (*Bungarus caeruleus*) samples used in this study, and their location information.

Sample ID	Locality	Latitude	Longitude
BC-1	Jallo Park, Lahore, Punjab, Pakistan	31°34'17.29"N	74°28'36.78"E
BC-2	Balochanwali, Bahawalpur, Punjab, Pakistan	29°28'56.90"N	71°59'41.86"E
BC-3	Qila Ram Qaur, Hafiz Abad, Punjab, Pakistan	32°4'57.12"N	73°40'49.02"E
BC-4	Yazman Housing Society, Yazman, Punjab, Pakistan	29°7'3.00"N	71°45'6.73"E
BC-5	Changa Manga Forest, Kasur, Punjab, Pakistan	31°4'54.19"N	73°59'53.49"E
BC-6	Lal Suhanra National Park, Bahawalpur, Punjab, Pakistan	29°19'1.36"N	71°54'16.43"E
BC-7	Rahim Yar Khan Zoo, Rahim Yar Khan, Punjab, Pakistan	28°24'14.30"N	70°15'32.63"E
BC-8	Chak Risalwala, Faisalabad, Punjab, Pakistan	31°22'4.90"N	73°1'24.80"E
BC-9	Qila Ram Qaur, Hafizabad, Punjab, Pakistan	32°4'57.12"N	73°40'49.02"E
BC-10	New City Housing Society, Jaranwala, Punjab, Pakistan	31°19'16.60"N	73°23'21.44"E
BC-11	Chak 126 GB Pind Janjua, Jaranwala, Punjab, Pakistan	31°21'38.48"N	73°25'28.74"E
BC-12	Rahim Yar Khan Zoo, Rahim Yar Khan, Punjab, Pakistan	28°24'14.30"N	70°15'32.63"E
BC-13	Yazman Housing Scheme, Yazman, Punjab, Pakistan	29°6'54.39"N	71°45'17.40"E
BC-14	Ayub National Park, Jehlam Road, Punjab, Pakistan	33°34'19.00"N	73°4'59.00"E
BC-15	Tibbi Balochan, Sadiqabad, Punjab, Pakistan	28°16'35.01"N	70°8'6.58"E
BC-16	Maraghzar Colony, Lahore, Punjab, Pakistan	31°30'8.71"N	74°14'55.48"E
BC-17	Lahore Zoo, Punjab, Pakistan	31°33'23.78"N	74°19'33.73"E
BC-18	Lahore Zoo, Punjab, Pakistan	31°33'23.78"N	74°19'33.73"E
BC-19	Raza Garden Phase 1, Sargodha, Punjab, Pakistan	32°2'51.23"N	72°37'31.68"E
BC-20	Pir wala, Jhang, Punjab, Pakistan	31°1'42.61"N	72°16'45.51"E
BC-21	Noor Garden, Okara, Punjab, Pakistan	30°48'48.38"N	73°28'38.33"E
BC-22	Chenab Park, Multan, Punjab, Pakistan	30°4'29.90"N	71°18'51.93"E
BC-23	Kalarwala, Chiniot, Punjab, Pakistan	31°28'26.21"N	72°33'56.11"E
BC-24	Qadir Abad Tiba, Sadiqabad, Punjab, Pakistan	28°16'54.33"N	70°7'45.48"E
BC-25	Chenab Park, Gujranwala, Punjab, Pakistan	30°4'29.90"N	71°18'51.93"E

tail tip biopsies were obtained from each specimen. After DNA extraction (Sambrook and Russel 2001), the Polymerase Chain Reaction (PCR) primers of representative mitochondrial genes (ND4, Cytochrome b, 12S rRNA, and 16S rRNA) and nuclear genes

(C-mos, RAG1, and NT3) from previous studies were used for the amplification of selected regions through PCR (see Table 2). After amplification, amplicons were sequenced bi-directionally by Big Dye™ Terminator on an ABI 3130XL Genetic analyzer. Forward and

Table 2. Mitochondrial and nuclear protein coding gene primers for Common Krait (*Bungarus caeruleus*).

Sr. No	Gene Name	Primer Sequence	Source
1	Cyt.b	5'-TGACTTGAARAACCAAYCGTTG-3'	Palumbi 1996
		5'-TGAGAAGTTTTTCYGGGTCRTT-3'	Parkinson et al. 2002
2	16S rRNA	5'-CGCCTGTTTAYCAAAAACAT-3	Vences et al. 2005
		5'-CCGGTCTGAACTCAGATCACGT-3'	Vences et al. 2005
3	12S rRNA	5'GTACACTTACCTTGTTACGACTT 3'	Knight and Mindell 1993
		5' AAAGTGGGATTAGATACCCCACTAT3'	Knight and Mindell 1993
4	ND4	5'-CATTACTTTTACTTGGATTGTCACCA-3'	Arevalo 1994
		5'-CACCTATGACTACCAAAAGCTCATGTAAGC-3'	Arevalo 1994
5	RAG-1	5'AGCTGCAGYCARTAYCAYAARATGTA3'	Chiari et al. 2004
		5'AACTCAGCTGCATTKCCAATRTCA3'	Chiari et al. 2004
6	NT3	5'ATATTTCTGGCTTTTCTCTGTGGC3'	Townsend et al. 2008
		5'GCGTTTCATAAAAATATTGTTTGACC3'	Townsend et al. 2008
7	C-mos	5' CATGGACTGGGATCAGTTATG 3'	Lawson et al. 2005
		5'CCTTGGGTGTGATTTTCTCACCT 3'	Lawson et al. 2005

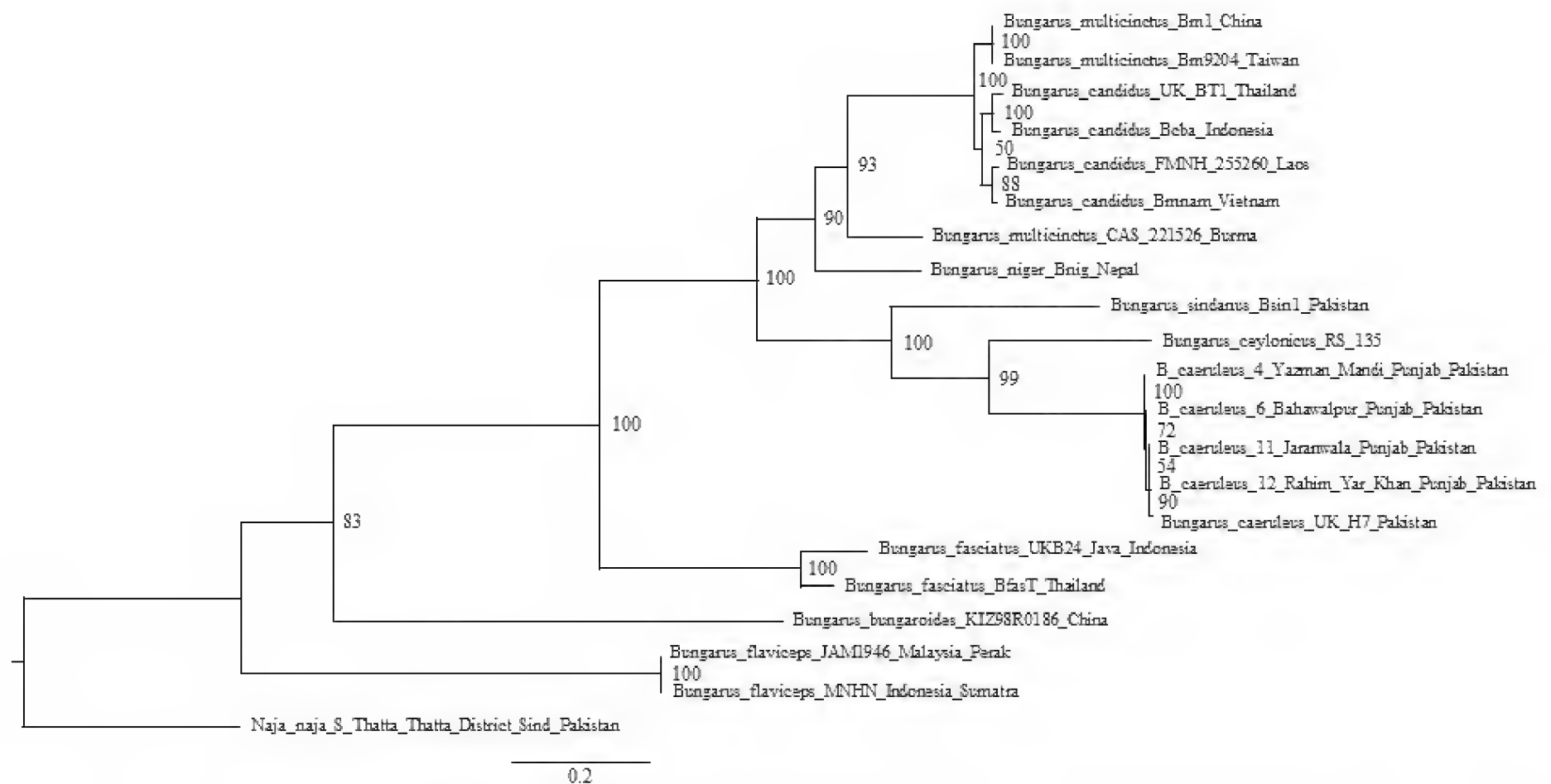


Fig. 2. Mitochondrial and nuclear genes (ND4, Cyt. b, COI, 12S rRNA, 16S rRNA, C-mos, RAG-1, NT3, and BDNF) based Maximum Likelihood phylogeny for Common Krait (*Bungarus caeruleus*).

reverse sequences were assembled through Sequencher 5.0 software. The resulting contigs (sequences) were given specific identities. These contigs were then aligned with other reported sequences obtained from the NCBI database through MEGA (v 6.0, Tamura et al. 2013) using the ClustalW tool for further data analyses. The nucleotide data for each gene were concatenated using SequenceMatrix (v1.7.8, Vaidya et al. 2011) software. The concatenated data were partitioned through PartitionFinder (v1.1.1, Lanfear et al. 2012) to give the best partition scheme and evolutionary models for phylogenetic analyses.

Two types of phylogenetic analyses, i.e., Maximum Likelihood (ML) and Bayesian inference (BI), were performed through RaxML (v8.0, Stamatakis 2014) and MrBayes (v3.2, Ronquist and Huelsenbeck 2012) software. The resulting phylogenetic trees were visualized and saved using Figtree (v1.4.3, <http://tree.bio.ed.ac.uk/software/figtree/>) software. DnaSP (v5.0, Librado and Rozas 2009) was used for analyzing polymorphic sites and DNA polymorphism, to determine the variation and genetic biodiversity in Common Krait (*Bungarus caeruleus*) in relation to other species of the genus *Bungarus*. Percent identity matrices were also constructed by comparing different species of every snake genus using online tool MUSCLE (available from the European Molecular Biology Laboratory, <https://www.ebi.ac.uk/Tools/msa/muscle/>).

Results

DnaSP software was used for analyzing the polymorphism of the mitochondrial and nuclear genes as shown in Table 3. The ribosomal RNA coding genes showed the least

variation, with lower numbers of variable sites, mutations, and parsimony informative sites. The polymorphism data show the variations in different mitochondrial and nuclear genes with their conservation among Common Krait and other species of genus *Bungarus*. In addition, no significant variations were found among Common Kraits from different cities in Pakistan.

The online MUSCLE tool was used to find relationships among *Bungarus* species on the basis of homology in the mitochondrial and nuclear genes (Table 4). This table shows the conservation patterns in mitochondrial and nuclear protein coding genes of various *Bungarus* species.

Phylogenetic analysis of Common Krait (*Bungarus caeruleus*) from Pakistan was conducted using mitochondrial and nuclear protein coding genes. In this study, Black Cobra (*Naja naja*) from Thatta Sindh was used as the outgroup for constructing maximum likelihood and Bayesian phylogenies. The best partition scheme and evolutionary models were used to infer the phylogenetic relationships of Common Krait in Pakistan with other members of genus *Bungarus* around the world. Concatenated Maximum likelihood and Bayesian Inference results gave very similar phylogenies (Figs. 2–3). All *Bungarus* species were divided into four main clades. The first clade included *B. candidus* (Indonesia, Thailand, Vietnam, and Laos), *B. multicinctus* (China, Taiwan, and Burma), and *B. niger* (Nepal). The second clade included *B. sindanus* and *B. caeruleus* (Pakistan), and *B. ceylonicus* (Sri Lanka). The third clade included *B. fasciatus* (Thailand and Indonesia), while the fourth clade included *B. bungaroides* (China) and *B. flaviceps* (Malaysia and Indonesia). The first and second clades showed a sister clade relationship with strong support (ML BS = 100, BI PP = 1). *Bungarus candidus* and

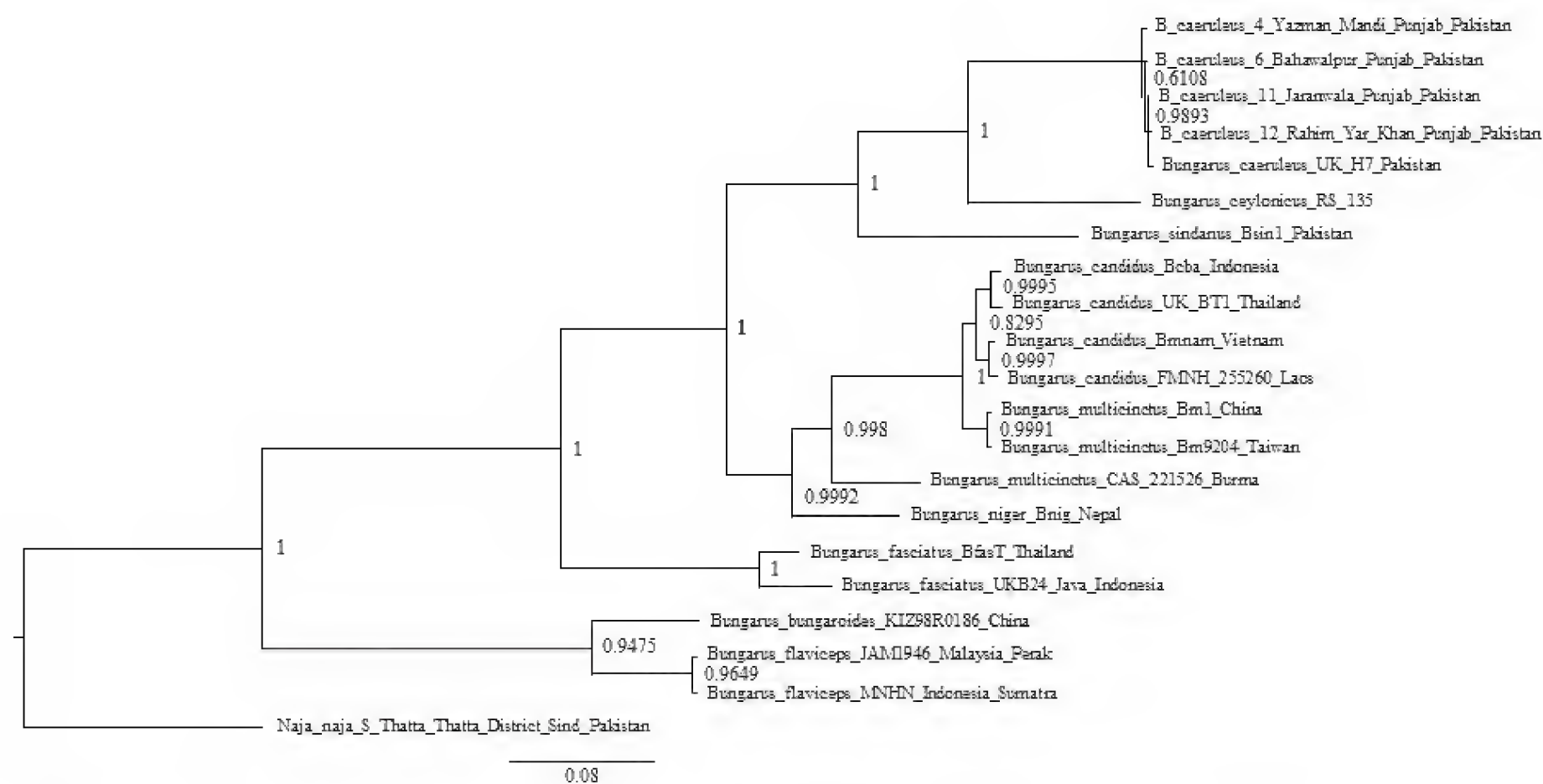


Fig. 3. Mitochondrial and nuclear genes (ND4, Cyt b, COI, 12S rRNA, 16S rRNA, C-mos, RAG-1, and NT3) Bayesian phylogeny for Common Krait (*Bungarus caeruleus*).

B. multicinctus probably diverged as separate species only recently. *Bungarus candidus*, *B. multicinctus*, and *B. niger* showed highly supported sister relationships with a complex pattern of divergence (BI PP = 1.0 ML BS = 90). In the second clade *B. sindanus* and *B. caeruleus* have been reported from Pakistan, thus they are sympatric species, while *B. ceylonicus* (from Sri Lanka) also showed a significant difference with strong support through Maximum likelihood and Bayesian inference phylogenies (PP = 1.0 and BS = 100). Pyron et al. (2012) also revealed the same relationships between *B. caeruleus*, *B. sindanus*, and *B. ceylonicus*. In addition to the molecular data obtained, examination of the 25 *B. caeruleus* specimens showed varying numbers of ventral scales (207–218), 15 rows of mid-body scales, and average numbers of subcaudals of 41–47.

Discussion

Elapids comprise 300 of the 2,500 known species of snakes (Leviton et al. 2018). The Southern Asian

elapids include cobras (*Naja* and *Ophiophagus*), kraits (*Bungarus*), long-glanded snakes (*Maticora*), and Asian coral snakes (*Calliophis*) [Sanz et al. 2019]. The uncertain phylogenetics of elapids has been a major factor for the varying numbers of identified species of elapids in the past (Mirtschin et al. 2017). This study aimed to characterize the genetic biodiversity and phylogenetic relationships of Common Krait (*Bungarus caeruleus*) as there is a great deal of unpublished data on this species. Here, mitochondrial and nuclear protein coding genes were used to construct the phylogeny of Common Krait from Pakistan along with some morphological characterization. One variable character is the number of ventral scales that ranges from 207–218 among the 25 specimens in this study. Khan (1985) wrote a note on the taxonomic status of Common Krait and Sindh Krait (*B. sindanus*), and by comparing 46 specimens, Khan noted an almost similar range of 207–218 ventral scales. The *B. caeruleus* in this study had 15 rows of mid-body scales which is the same as described by Khan (1985). *Bungarus caeruleus* and *B.*

Table 3. Polymorphism in mitochondrial and nuclear protein coding genes of Common Krait (*Bungarus caeruleus*).

Parameters	ND4	Cytochrome b	12S rRNA	16S rRNA	C-mos	RAG-1	NT3
Total number of sites	619	702	650	520	586	802	425
Variable number of sites	302	325	270	191	419	665	325
Number of mutations	302	202	63	47	12	20	32
Singleton variable sites	43	51	20	26	09	19	03
Parsimony informative sites	196	151	43	21	03	01	28
Segregating sites	159	151	00	00	12	19	31
Synonymous changes	176	156	00	00	06	03	23
Number of haplotypes	17	16	08	10	04	03	05
Haplotype diversity	0.866	0.615	0.686	0.521	0.333	0.145	0.754
Nucleotide diversity	0.08243	0.09528	0.03617	0.03451	0.00288	0.00226	0.03389

Table 4. Percent homology of mitochondrial and nuclear genes for Common Krait (*Bungarus caeruleus*) from Pakistan among various *Bungarus* species and countries, sorted by decreasing Cytb homology. The asterisk (*) indicates the sequence from this study, all others are based on GenBank sequences.

Species	Country	Homology percentages of nine representative genes								
		Cytb	ND4	12S	16S	COI	C-mos	RAG-1	NT3	BDNF
<i>B. caeruleus</i> *	Pakistan	100	100	100	100	100				
<i>B. caeruleus</i>	Pakistan	99.84	99.51	100	100	99.99				
<i>B. ceylonicus</i>	NA	89.21	87.46	93.68	96.62		100	99.4		
<i>B. candidus</i>	Indonesia	89.19	84.53	90.57	95.08					
<i>B. niger</i>	Nepal	86.31	84.36							
<i>B. sindanus</i>	Pakistan	86.31	85.5							
<i>B. candidus</i>	Thailand	85.35	83.88							
<i>B. multicinctus</i>	Burma	85.19	84.34			85.65				
<i>B. multicinctus</i>	China	85.19	85.18							
<i>B. candidus</i>	Vietnam	85.19	85.67							
<i>B. multicinctus</i>	Taiwan	85.02	85.5	91.73	90.09	86.11				
<i>B. fasciatus</i>	Thailand	83.74	83.55	90.79	91.91					
<i>B. fasciatus</i>	Indonesia	83.57	83.39	91.38	95.5	85.03	99.38	99.62	96.63	99.76

sindanus had 15 and 17 rows of mid-body scales, with the central larger row being hexagonal and white in color. Boulenger (1897) also observed 15 rows of mid-body scales in Indo-Pakistan Common Krait while 17 mid-body scale rows were reported in *B. sindanus* from Indus Basin. The average number of sub-caudals observed here is 41–47 which is within the range observed by Khan (1985): 40–54 in males and 30–54 in females. Boulenger (1897) reported small eyes with round pupil which is similar to those observed in this study.

There are also reports about the distribution of *B. caeruleus* in Indo-Pakistan subcontinent. Eastward it is found in Assam and Bengal (Jamal et al. 2018; Ganesh and Vogel 2018); westward to the Pakistan-Iran Border; Shockley (1949) and Kral (1969) reported it in Afghanistan; Smith (1943) reported it southward in Peninsular India and the Andaman Islands; and de Silva (1981) also reported it in Sri Lanka. This study is one attempt to infer the phylogenetics of *B. caeruleus* in Pakistan, but suggests more studies from the above-mentioned parts of the *B. caeruleus* distribution are needed, as there are almost no studies from other parts of of the Indo-Pakistan subcontinent on the phylogenetics of the Common Krait *B. caeruleus*.

Determining the relationships among the members of Elapidae can help in understanding the distribution and diversity of elapids. Many studies have focused on evolutionary relationships of elapids, and this study examined the molecular phylogenetics of *B. caeruleus* from Pakistan. The second clade (Figs. 2–3) includes *B. sindanus* and *B. caeruleus* (Pakistan), and *B. ceylonicus* (Sri Lanka). *Bungarus sindanus* and *B. caeruleus* are sympatric species, while *B. ceylonicus* also showed a significant difference with strong support through the Maximum likelihood and Bayesian inference phylogenies

(PP = 1.0 and BS = 100). Pyron et al. (2012) also revealed the same relationships between *B. caeruleus*, *B. sindanus*, and *B. ceylonicus*. They presented a large-scale phylogeny of squamate reptiles for future comparative studies, and a revised classification of squamates at the family and subfamily levels so that taxonomy might be brought in a line with data from the new phylogenetic studies. Their phylogeny shows the same relationship (ML BS = 100, BI = 1) between *B. caeruleus*, *B. sindanus*, and *B. ceylonicus* as is shown in this study through Maximum Likelihood and Bayesian phylogenies.

Conclusions

Most of the currently recognized krait species (genus *Bungarus*) are poorly understood. This study characterized the genetic biodiversity and phylogenetic relationships of Common Krait (*Bungarus caeruleus*) from Pakistan showing inter- and intra-specific variations among different geographical regions of the world. More diverse sampling and a larger number of samples with more genomic data could help to further resolve the taxonomic status of the *Bungarus* species in Pakistan. This study also provides guidance for the correct identification of these snakes with authentication using molecular biology tools which will be helpful in the development of effective and region-specific antivenoms for such venomous snakes.

Literature Cited

Arevalo E, Davis SK, Sites JW Jr. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae)

- in Central Mexico. *Systematic Biology* 43(3): 387–418.
- Blanquart F. 2019. Evolutionary epidemiology models to predict the dynamics of antibiotic resistance. *Evolutionary Applications* 12(3): 365–383.
- Boulenger GA. 1897. A new krait from Sind (*Bungarus sindanus*). *Journal of the Bombay Natural History Society* 11: 73–74.
- Chandrasekaran K, Anjaneyulu M, Choi J, Kumar P, Salimian M, Ho CY, Russell JW. 2019. Role of mitochondria in diabetic peripheral neuropathy: Influencing the NAD⁺-dependent SIRT1-PGC-1 α -TFAM pathway. *International Review of Neurobiology* 145: 177–209.
- Chiari Y, Vences M, Vieites DR, Rabemananjara F, Bora P, Ravoahangimalala OR, Meyer A. 2004. New evidence for parallel evolution of color patterns in Malagasy poison frogs (*Mantella*). *Molecular Ecology* 13: 3,763–3,774.
- Chippaux JP. 1998. Snake-bites: appraisal of the global situation. *Bulletin of the World Health Organization* 76: 515–524.
- da Silva WO, da Costa MJR, Pieczarka JC, Rissino J, Pereira JC, Ferguson-Smith MA, Nagamachi CY. 2019. Identification of two independent X-autosome translocations in closely related mammalian (*Proechimys*) species. *Scientific Reports* 9(1): 4,047.
- De Silva A. 1981. Snakebites in Anuradhapura district. *The Snake* 13: 117–130.
- Friberg M, Schwann C, Guimarães PR, Raguso RA, Thompson JN. 2019. Extreme diversification of floral volatiles within and among species of *Lithophragma* (Saxifragaceae). *Proceedings of the National Academy of Sciences of the United States of America* 116(10): 4,406–4,415.
- Ganesh SR, Vogel G. 2018. Taxonomic reassessment of the Common Indian Wolf Snakes *Lycodon aulicus* (Linnaeus, 1758) complex (Squamata: Serpentes: Colubridae). *Bonn Zoological Bulletin* 67(1): 25–36.
- Grismer LL, Davis HR. 2018. Phylogeny and biogeography of Bent-toed Geckos (*Cyrtodactylus* Gray) of the Sundaic swamp clade. *Zootaxa* 4472(2): 365–374.
- Hoffstetter R, Gasc JP. 1969. Vertebrae and ribs of modern reptiles. Pp. 201–310 In: *Biology of the Reptilia. Volume 1: Morphology*. Editors, Gans C, Bellairs A, Parsons TS. Academic Press, London, United Kingdom. 373 p.
- Jamal Q, Idrees M, Ullah S, Adnan M, Zaidi F, Zaman Q, Rasheed SB. 2018. Diversity and altitudinal distribution of Squamata in two distinct ecological zones of Dir, a Himalayan sub-zone of northern Pakistan. *Pakistan Journal of Zoology* 50(5): 1,835–1,839.
- Kester L, van Oudenaarden A. 2018. Single-cell transcriptomics meets lineage tracing. *Cell Stem Cell* 23(2): 166–179.
- Khan MS. 1985. Taxonomic notes on *Bungarus caeruleus* (Schneider) and *Bungarus sindanus* Boulenger. *The Snake* 17: 71–78.
- Khan MS. 2002a. *Die Schlangen Pakistans*. Edition Chimaira, Frankfurt am Main, Germany. 265 p.
- Khan MS. 2002b. *A Guide to the Snakes of Pakistan*. Edition Chimaira, Frankfurt am Main, Germany. 265 p.
- Knight A, Mindell DP. 1993. Substitution bias, weighting of DNA sequence evolution, and the phylogenetic position of Fea's Viper. *Systematic Biology* 42: 18–31.
- Kral B. 1969. Notes on the herpetofauna of certain provinces of Afghanistan. *Zoologische Listy* 18: 55–66.
- Lanfear R, Calcott B, Ho SY, Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1,695–1,701.
- Lawson R, Slowinski JB, Crother BI, Burbrink FT. 2005. Phylogeny of the Colubroidea (Serpentes): new evidence from mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* 37: 581–601.
- Leviton AE, Siler CD, Weinell JL, Brown RM. 2018. Synopsis of the snakes of the Philippines. *Proceedings of the California Academy of Sciences* 64(14): 399–568.
- Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25(11): 1,451–1,452.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 In: *2010 Gateway Computing Environments Workshop*. Institute of Electrical and Electronics Engineers, Piscataway, New Jersey, USA.
- Mirtschin P, Rasmussen A, Weinstein S. 2017. *Australia's Dangerous Snakes: Identification, Biology and Envenoming*. CSIRO Publishing, Melbourne, Australia. 432 p.
- Ng YS, DiMauro S, Turnbull DM. 2019. Mitochondrial medicine: a historical point of view. Pp. 1–18 In: *Diagnosis and Management of Mitochondrial Disorders*. Editors, Mancuso M, Klopstock T. Springer, Cham, Switzerland. 382 p.
- Nisar A, Rizvi F, Afzal M, Shafi MS. 2009. Presentation and complications of snakebite in a tertiary care hospital. *Journal of College of Physicians and Surgeons Pakistan* 19: 304–307.
- Oh AMF, Tan CH, Tan KY, Quraishi NH, Tan NH. 2019. Venom proteome of *Bungarus sindanus* (Sind krait) from Pakistan and in vivo cross-neutralization of toxicity using an Indian polyvalent antivenom. *Journal of Proteomics* 193: 243–254.
- Palumbi SR. 1996. Nucleic acids II: the polymerase chain reaction. Pp. 205–247 In: *Molecular Systematics*. 2nd Edition. Editors, Hillis DM, Moritz C, Mable BK. Sinauer Associates, Sunderland, Massachusetts, USA. 665 p.
- Parkinson CL, Campbell JA, Chippendale PT. 2002. Multigene phylogenetic analysis of pitvipers with comments on the biogeographical history of the

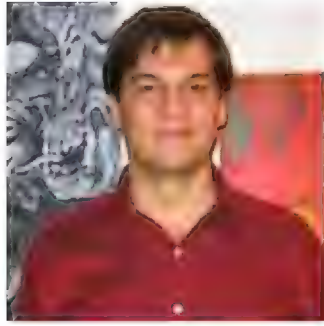
- group. Pp. 93–110 In: *Biology of the Vipers*. Editors, Schuett GW, Hoggren M, Douglas ME, Greene HW. Eagle Mountain Publishing, Utah, USA. 580 p.
- Pilfold NW, Letoluai A, Ruppert K, Glikman JA, Stacy-Dawes J, O'Connor D, Owen M. 2019. Confirmation of black leopard (*Panthera pardus pardus*) living in Laikipia County, Kenya. *African Journal of Ecology* 57(2): 270–273.
- Pyron RA, Burbrink FT. 2012. Extinction, ecological opportunity, and the origins of global snake diversity. *Evolution* 66: 163–178.
- Pyron RA, Burbrink FT, Wiens JJ. 2013. A phylogeny and revised classification of Squamata, including 4,161 species of lizards and snakes. *BMC Evolutionary Biology* 13: 93.
- Reeder TW, Townsend TM, Mulcahy DG, Noonan BP, Wood Jr PL, Sites Jr JW, Wiens JJ. 2015. Integrated analyses resolve conflicts over squamate reptile phylogeny and reveal unexpected placements for fossil taxa. *PLOS One* 10: e0118199.
- Roland B. 1994. *Snakes: A Natural History*. Sterling Publishing, New York, New York, USA. 220 p.
- Ronquist F, Huelsenbeck JP. 2012. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1,572–1,574.
- Sambrook J, Russel DW. 2001. Rapid isolation of yeast DNA. Pp. 631–632 In: *Molecular Cloning, a Laboratory Manual*. Editors, Sambrook J, Russel, DW. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA. 721 p.
- Sanz L, Quesada-Bernat S, Ramos T, Casais-e-Silva LL, Corrêa-Netto C, Silva-Haad JJ, Calvete JJ. 2019. New insights into the phylogeographic distribution of the 3FTx/PLA2 venom dichotomy across genus *Micrurus* in South America. *Journal of Proteomics* 200: 90–101.
- Shockley CH. 1949. Herpetological notes for Ras Jiunri, Baluchistan. *Herpetologica* 5: 121–123.
- Smith MA. 1943. *The Fauna of British India including Ceylon and Burma*. Taylor and Francis, London, United Kingdom. 185 p.
- Soroka M, Burzyński A. 2018. Doubly uniparental inheritance and highly divergent mitochondrial genomes of the freshwater mussel *Unio tumidus* (Bivalvia: Unionidae). *Hydrobiologia* 810(1): 239–254.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1,312–1,313.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 12: 2,725–2,729.
- Townsend TM, Alegre RE, Kelly ST, Wiens JJ, Reeder TW. 2008. Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: an example from squamate reptiles. *Molecular Phylogenetics and Evolution* 47: 129–142.
- Vaidya G, Lohman DJ, Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27: 171–180.
- Vences M, Thomas M, Bonett RM, Vieites DR. 2005. Deciphering amphibian diversity through DNA barcoding: chances and challenges. *Philosophical Transactions of the Royal Society of London B. Biological Sciences* 360(1462): 1,859–1,868.
- Yulin X, Ping W, Guanghui Z. 2018. Molecular phylogeny found the distribution of *Bungarus candidus* in China (Squamata: Elapidae). *Zoological Systematics* 43(1): 109–117.
- Zhou P, Fan H, Lan T, Yang XL, Shi WF, Zhang W, Zheng XS. 2018. Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature* 556(7700): 255.



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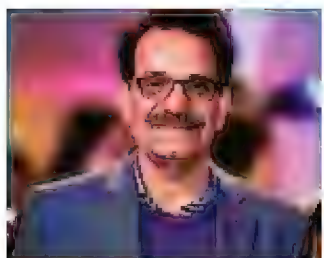
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Daboia russelii (Reptilia: Squamata) in remote parts of Gujjar Village Miandam, Swat, Khyber Pakhtunkhwa, Pakistan

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Abstract.—Snakes are widely perceived with fear by the general public in Pakistan, and they are often killed on sight. The present study examines the range extension of *Daboia russelii* in remote parts of Gujjar village Miandam Swat, Pakistan. Seven snakes were collected, including three which were attacked and injured by the local men, and four others observed in the natural habitats in four localities: Karoo, Kalandori, Chharr, and Dhop, from June to September in both 2016 and 2017. Morphometric analysis, details of the coloration, and photographs of the snakes are provided. Russell's Vipers were seen frequently in grasslands, cultivated fields, and areas near human residences. These snakes were mostly seen after sunset. This species has also been reported from other parts of Pakistan, but the present records represent a new locality.

Keywords. Russell's Viper, Viperidae, morphometric analysis, range extension, snake, venomous

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Snakes are the most reviled of vertebrates, widely perceived by the public as dangerous and harmful. The 2,700 species of snakes known to science include 375–400 venomous species, of which approximately 200 species are considered life-threatening to man and other animals. Snakes of the families Elapidae, Crotalidae, and Viperidae are venomous (Durand 2004; Vidal et al. 2009).

Russell's Viper is named as *Daboia russelii* in honor of Patrick Russell (1726–1825). *Daboia russelii* is an infamous venomous snake of the Old World, found in Bangladesh, Cambodia, China, Indonesia, Myanmar, Nepal, Pakistan, Sri Lanka, Taiwan, and Thailand (McDiarmid et al. 1999). In Pakistan it occurs from the Indus Valley to the Kashmir, and east to Bengal. This snake is frequently found in Thatta District, Sindh, and at low elevations in Punjab, but no published report is yet available from the northwestern parts of Pakistan.

Relatively few studies have been conducted on the fauna of Swat, and even fewer studies are associated with the reptilian fauna of the region (Smith 1943; Minton 1962, 1965; Mertens 1969, 1970; Khan 1982, 1984, 1985). More than fifty species of terrestrial snakes are known from Pakistan (Khan 1980, 1982, 1997). Khan (2002) conducted an extensive survey of different climatic zones in Pakistan for herpetofaunal diversity.

The present study reports the existence of Russell's Vipers in the hilly areas of Swat Valley, Pakistan.

Village council Miandam is located at 35°03'12"N, 72°33'39"E, about 57 km from Saidu Sharif, District Swat, Khyber Pakhtunkhwa, at an elevation of approximately 1,918 m asl. The study area (Fig. 1) falls under moist temperate forest, thus receiving summer monsoon and winter snow fall. Because of its cool climate and green hillsides, the area is frequented by tourists (Forest Working Plan 2013).

In village council Miandam, there are two sub-villages, namely Gujjar village and Swati village. Four sites of the Gujjar village were surveyed from June to September in 2016 and 2017 (Fig. 2). The snake specimens (Table 1) were either collected dead following their attack by the local people, or recorded with visual observations using the method of Campbell and Christman (1982).

The photographs of specimens shown here (Fig. 3) were taken using a Nikon Coolpex L330 camera. Morphometric analysis of the snake specimens collected dead were recorded using a digital caliper (Precision 145). The specimens observed were identified with the help of keys provided by Khan (2002). The general characteristics of *Daboia russelii* specimens from the four localities of Gujjar village Miandam, Swat (2016–2017) are as follows:

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Fig. 1. Map of Khyber Pakhtunkhwa, red circle shows the study area in the District Swat within the province.

Table 1. Records of the seven specimens observed by month and village.

	2016				2017			
	Dhop	Chharr	Karoo	Kalandori	Dhop	Chharr	Karoo	Kalandori
January								
February								
March								
April								
May								
June		live						
July	dead							
August			dead		live		live	dead
September						live		
October								
November								
December								

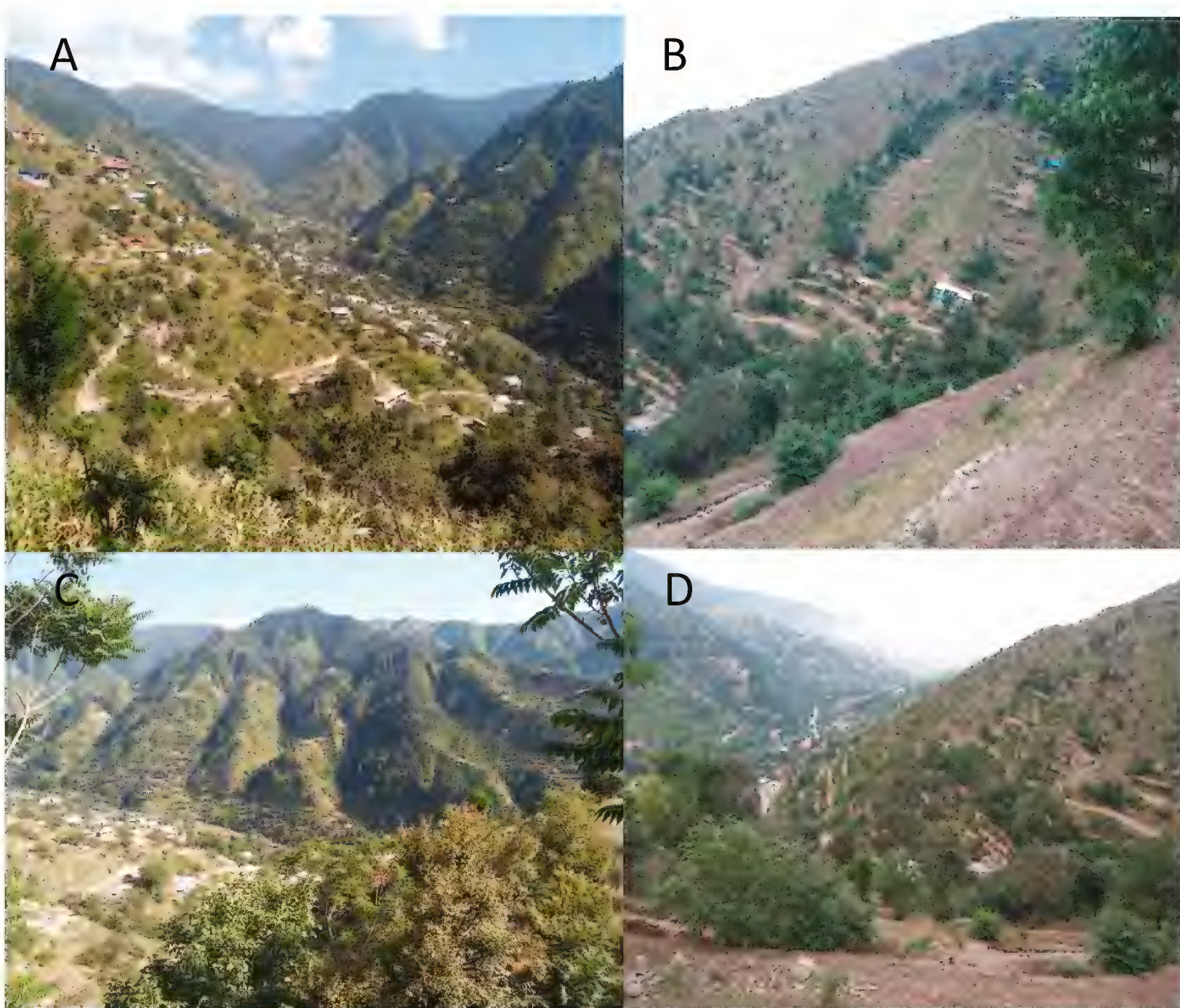


Fig. 2. Collection sites of *Daboia russelii* in Gujjar village Miandam, Swat, KP, Pakistan. (A) Karoo, 35°3'32"N 72°33'11"E; (B) Kaalandori, 35°3'31"N 72°32'21"E; (C) Chhar 35°3'34"N 72°33'12"E; (D) Doop, 35°3'23"N 72°32'58"E.

- **Head:** Longer rather than broad, distinctly wider than neck.
- **Body:** Stout, flattened dorsoventrally, tapering evenly both posteriorly and anteriorly.
- **Snout:** Bluntly pointed, snout-vent length 1,022–1,075 mm, tail 215–223 mm.
- **Rostral:** About twice as high as wide.
- **Nostril:** Large, crescent shaped in large nasal scale.
- **Supraocular scale:** Entire, not divided.
- **Supralabials:** 12, separated from eye by three or four rows of small scales.
- **Infralabials:** 14.
- **Anterior chin shield:** Short and wide, posterior not well differentiated from surrounding scales.
- **Dorsal scales:** Keeled except for lowest row, 29–31 rows at mid-body, reduction posteriorly to 23 or 21 rows, usually an anterior reduction of two or four rows.
- **Ventrals:** 165–173.
- **Total body length:** 76.2 cm, tail length 15.2 cm (16% of total body length).
- **Coloration:**
 - Dorsal ground color light tan to sandy.
 - Chest net spots with black or dark brown

borders and edge creamy, these spots fused to a greater or lesser extent, lateral series of similar but smaller spots below which are scattered dark flecks with light edges.

- Two large dark spots at base of head.
- A light V-shaped mark with its apex on top of snout.
- Labial sides of snout mottled with brown and cream.
- Belly whitish with black semilunar spots.
- Chin or throat white.
- Many scales topped with black.

The fauna and flora of Pakistan is Oriental, Palearctic, Ethiopian, and Central Asian in nature, with many endemic forms (Smith 1931; Khan 1980). In Pakistan the complex of habitats is diverse, including oceans, swamps, rivers, lakes, flood plains, arid plains, sand and rocky deserts; tropical thorn, tropical dry deciduous, subtropical dry, subtropical arid, subtropical pine, dry and moist temperate subalpine forests; grassy tundra and cold deserts. Moreover, most of the habitats are now heavily influenced by anthropogenic activities which negatively affect the fauna and flora of the country (Baig 1975).

The present study describes seven specimens of



Fig. 3. Photos of the dead *Daboia russelii* specimens from Gujjar village Miandam, Swat, KP, Pakistan. (A, B) Dorsal views, (C, D) Fangs, (E) Black spots on ventral side, (F) Anal orifice with tail showing a zip-like structure.

Russell's Vipers from four localities in the study area, including two specimens each from Dhoop, Chhar, and Karoo, and one specimen from Kalandori Gujjar Village Miandam, Swat, Pakistan. Russell's Viper is one of the most widespread of Asiatic venomous snakes. While surveying the literature no published records for this species are available in Swat Valley, Pakistan. Therefore, the present study documents the presence of this species in the hilly areas of Swat Valley.

Literature Cited

- Baig AR. 1975. Wild life habitats of Pakistan. Biological Science Research Desert, Punjab, Pakistan. *Journal of the Bombay Natural History Society* 82: 144–148.
- Campbell HW, Christman SP. 1982. Field techniques for herpetofaunal community analysis. *Wildlife Research Report* 13: 193–200.
- Durand JF. 2004. The origin of snakes. Pp. 187 In: *Geoscience Africa 2004*. University of the Witwatersrand, Johannesburg, South Africa.
- Forest Working Plan. 2013–2014. Forestry Sector Project, Village Plan, Miandam, Pakistan. pp. 7–13.
- Khan MS. 1980. Affinities and zoogeography of herptiles of Pakistan. *Biologia* 26: 113–171.
- Khan MS. 1982. An annotated checklist and key to the reptiles of Pakistan. Part III: Serpentes (Ophidia). *Biologia* 28(2): 215–254.
- Khan MS. 1984. Validity of the natricine taxon *Xenochrophis sanctijohannis* Boulenger. *Journal of Herpetology* 18: 198–200.
- Khan MS. 1985. *An Interesting Collection of Amphibians and Reptiles from Cholistan Division*. Bulletin Number 5. Botany Department, Pakistan Forest

- Institute, Peshawar, Pakistan.
- Khan MS. 1997. A new toad of genus *Bufo* from the foot of Siachin Glacier, Baltistan, northeastern Pakistan. *Pakistan Journal of Zoology* 29: 43–48.
- Khan MS. 2002. *A Guide to the Snakes of Pakistan*. Edition Chimaira, Frankfurt am Main, Germany. 265 p.
- Mertens R. 1969. Die Amphibien und Reptilien West-Pakistans. *Stuttgarter Beiträge zur Naturkunde* 197: 1–96.
- Mertens R. 1970. Die Amphibien und Reptilien West-Pakistans. *Stuttgarter Beiträge zur Naturkunde* 216: 1–5.
- Minton SA, Anderson S. 1965. A new dwarf gecko (*Tropicolotes*) from Balochistan. *Herpetological Bulletin* 21: 59–61.
- Minton SA. 1962. An annotated key to the amphibians and reptiles of Sind and Las Bela, West Pakistan. *American Museum Novitates* 81: 1–21.
- Smith MA. 1931. *The Fauna of British India, including Ceylon and Burma. Reptilia and Amphibia*. Volume I. Taylor and Francis, London, United Kingdom. 85 p.
- Smith MA. 1943. *The Fauna of British India, Ceylon and Burma. Reptilia and Amphibia*. Volume III. Taylor and Francis, London, United Kingdom. 583 p.
- Vidal N, Rage JC, Couloux A, Hedges SB. 2009. Snakes (serpents). *The Time Tree of Life* 23: 390–397.



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Native anuran species as prey of invasive American Bullfrog, *Lithobates catesbeianus*, in Brazil: a review with new predation records

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Abstract.—The American Bullfrog (*Lithobates catesbeianus*) is widely distributed throughout the world as an invasive species, and causes negative impacts on the fauna resulting from its voracious predatory activity. This study documents two new predation reports and reviews the previous predation reports of the American Bullfrog on native Brazilian anurans. Twenty-one species of native anurans were recorded as American Bullfrog prey in Brazil. A positive correlation was found between the number of native anurans preyed on by American Bullfrog and the respective family or number of species per genus. Most of the prey species are small or medium-sized, and the results suggest that the generalist diet and intraguild predation may have favored the widespread establishment of the American Bullfrog.

Keywords. Amphibia, Atlantic Forest, biological invasion, conservation, global change, intraguild predation, exotic species

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Introduction

Biological invasions represent a major threat to natural ecosystems and their respective biodiversity, human health, and food security (IUCN 2012). In this context, the American Bullfrog, *Lithobates catesbeianus* (Shaw, 1802), is a globally widespread introduced species (Lowe et al. 2004). It is native in North America, occurring from eastern Canada and the central and eastern United States to northeastern Mexico (Quiroga et al. 2015). The introduction of *L. catesbeianus* in non-native

environments has direct (e.g., predation and competition) and indirect (e.g., parasites, disease introduction, and biotic homogenization) impacts on biodiversity (Batista 2002; Batista et al. 2015; Kiesecker and Blaustein 1998; Kraus 2009).

Lithobates catesbeianus is a voracious predator whose diet includes a wide variety of prey (Boelter and Cechin 2007; Boelter et al. 2012; Silva et al. 2009). Juveniles feed mainly on insects (Silva et al. 2009), whereas adults prey upon invertebrates and small vertebrates, such as fish, reptiles, birds, and mammals (Quiroga et al. 2015).

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American Bullfrogs are considered opportunistic feeders, also preying on amphibians, including conspecifics and other species (Silva et al. 2011; Toledo et al. 2007).

The American Bullfrog is now established in nearly 40 countries around the world (Frost 2019; Kraus 2009). In Brazil, the first specimens were introduced in 1935 for commercial exploitation at the municipality of Itaguaí, Rio de Janeiro state (Vizotto 1984). The introduction of the American Bullfrog for commercial frog farming was due to its fast reproduction and greater development in captivity compared to native species. It occurs mainly in the southern and southeastern Brazilian states because of its easy adaptation to the climatic conditions (Vizotto 1984). Approximately 2,000 commercial frog farms were active in the early 1990s in Brazil, but many closed their activities because of low profitability (Lima and Agostinho 1988), which led to American Bullfrog specimens being abandoned or released into the natural environments, and consequently several accidental invasions have occurred in Brazil (Both et al. 2011).

Populations of *Lithobates catesbeianus* are now known to be present in 155 Brazilian municipalities (Both et al. 2011; Instituto Horus 2016), a context in which many studies have revealed the localized impacts of its predatory activity on native anuran fauna (Batista et al. 2015; Boelter and Cechin 2007; Boelter et al. 2012; Leivas et al. 2012; Silva et al. 2011). In addition, global-scale studies have demonstrated trophic niche-width shifts in bullfrog populations from both native and invaded areas (Bissattini and Vignoli 2017), as well as the effects of the interactions between bullfrogs and crayfish on native amphibians (Bissattini et al. 2018, 2019; Liu et al. 2018). However, studies summarizing data on the predation of native anurans by American Bullfrogs have yet to be presented; therefore, knowledge on the impact and the native anuran species preyed upon by such an invasive frog may benefit our understanding of their predator-prey relationships.

Herein, the predation of *Boana raniceps* and *Phyllomedusa distincta* by males of *Lithobates catesbeianus* are reported, and the available literature on the predation of native anurans by the invasive frog *L. catesbeianus* in Brazil is reviewed. An overview on the number and identities of native species reported as prey and the potential impact of the American Bullfrog on native anurans are provided.

Material and Methods

Bibliographic Review

An extensive literature review was conducted to find scientific articles, natural history notes, and theses which contain reports on the predation of native anurans by the

invasive American Bullfrog *Lithobates catesbeianus* in Brazil. The sources included articles or natural history notes published in *Herpetological Review* (1967–2018), *Herpetological Bulletin* (2008–2018), *Herpetology Notes* (2008–2018), and *South American Journal of Herpetology* (2006–2018). Searches were also conducted in Web of Science using the following query: (“*Rana catesbeiana*” OR “*Lithobates catesbeianus*”) AND (“diet” OR “feeding biology” OR “predation”), applied in the field “topic” on 30 December 2018, without applying any filters for year or other parameters. Considering that predation attempts would not necessarily result in a predation event (Toledo et al. 2007), reports of predation attempts in the field, laboratory experiments, or captivity were not included. Masters and doctoral papers in digital format were obtained from the library databases of Brazilian universities (especially Universidade Estadual Paulista and Universidade Regional de Blumenau) by using the search terms mentioned above in the Google search engine.

The Web of Science query resulted in 159 studies, three of which met the criteria and were included in the study. Eight additional predation records were selected for inclusion in the study by searching the selected journals (six studies) and the library databases of Brazilian universities (two studies). Information was extracted from each diet analysis (i.e., the diet was described through the analyses of stomach contents or predation records), study location, anuran prey species, geographic range, and body size. The geographic range follows the list of anuran species for each Brazilian federal state and the biomes proposed in Toledo and Batista (2012). The body sizes of anuran species follow the size values available in Uetanabaro et al. (2008) and Haddad et al. (2013). The spatial distribution map of *Lithobates catesbeianus* invasive populations and predation reports were generated with 155 occurrence points for American Bullfrog in Brazil, obtained from Both et al. (2011) and Instituto Horus (2016).

Data Analysis

The relation between the number of native anuran species preyed upon by the American Bullfrog and the number of native anurans per family or genus was tested with a Pearson correlation analysis. The numbers of native anuran prey species per family and genus were compiled following the Frost (2019) database. Toledo et al. (2007) stated that a positive correlation between the number of predation events and taxonomic richness may be a proxy for search representativeness, by reasoning that taxa with more species would be more frequently preyed by chance (i.e., a sampling effect). Such a correlation could indicate the possible mechanisms of *L. catesbeianus* impacts on native biota apart from search representativeness.



Fig. 1. Adult *Lithobates catesbeianus* swallowing an adult *Boana raniceps* in an artificial permanent pond within pasture area in southern Brazil.

Results

Field Observations and New Predation Records

An adult *Lithobates catesbeianus* swallowing an adult *Boana raniceps* (Fig. 1) was recorded on 11 October 2014 at 2100 h, in an artificial permanent pond inside a pasture area (23°20'38"S, 51°52'07"W), in the northern region of Paraná state, southern Brazil. Although the specimens escaped, voucher specimens of the native anuran species and *L. catesbeianus* had been previously collected by Affonso et al. (2014) and stored at the Amphibian Collection from the Zoology and Botany Department, Bioscience Institute, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil.

A second predation event recorded a male adult specimen of Bullfrog swallowing a treefrog (Fig. 2A). The specimen was collected during an *L. catesbeianus* survey on 22 January 2019, at 2200 h, in an artificial permanent pond in a rural property at Iporanga, southern São Paulo state, southeastern Brazil (24°35'01.2"S, 48°36'00.4"W). The *L. catesbeianus* specimen was taken to the laboratory where the anuran prey was removed and identified as an adult *Phyllomedusa distincta* (Fig. 2B).

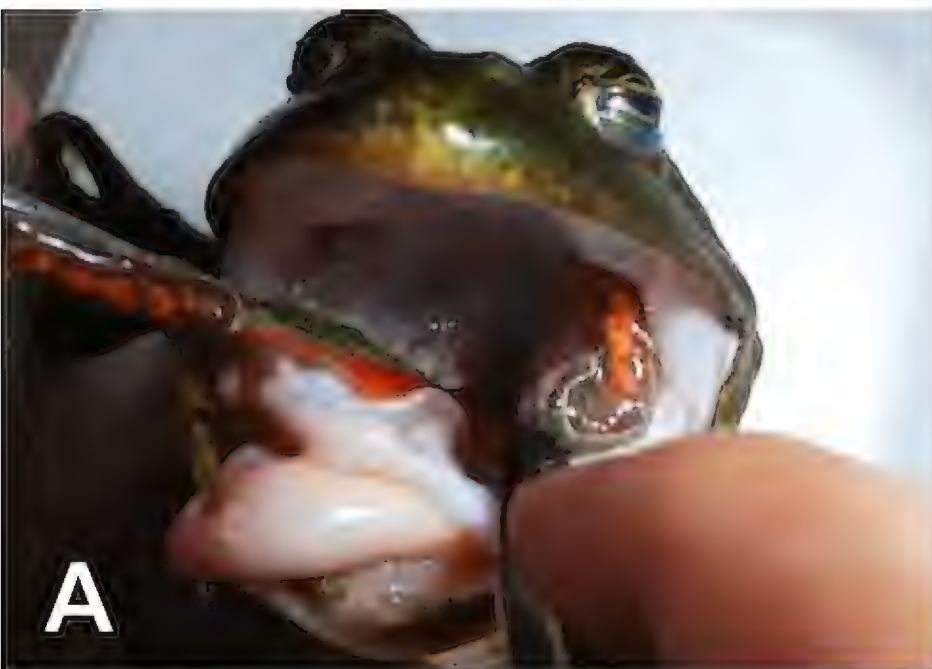


Fig. 2. (A) Predation of an adult *Phyllomedusa distincta* by *Lithobates catesbeianus*, (B) Adult *P. distincta* partially digested, removed from the oral cavity of *L. catesbeianus*.

Exploratory Analysis

Overall, 11 publications reported predation events, corresponding to 41 records of native anurans as prey of *L. catesbeianus* (Table 1). Nine of the publications discussed the diet in a broader sense, and two were natural history notes reporting predation events. Most of the records occurred in Minas Gerais state (39%), followed by Rio Grande do Sul (~32%), Paraná (~12%), São Paulo (~12%), and Santa Catarina (~5%), at sites inside the Atlantic Forest, in addition to another site in a transition zone between Cerrado and Atlantic Forest (Fig. 3, Table 1).

This survey accounted for 21 anuran species as prey of *L. catesbeianus*, all widely distributed and possibly coexisting with American Bullfrogs in their breeding sites. The anuran family Hylidae had the highest number of species (11 species), followed by Leptodactylidae (four species), Bufonidae and Microhylidae with two species each, and Odontophrynidae and Phyllomedusidae with one species each. *Lithobates catesbeianus* often preyed on medium-sized species, but small-sized species were also preyed upon (Table 1).

A positive correlation was found between the number of native anuran species preyed on by American Bullfrog and genus richness ($r = 0.71$, $P = 0.01$), whereas at the family level no relationship was found ($r = 0.53$, $P = 0.22$). Thus, considering the studies analyzed, genera with higher numbers of species presented more potential prey for American Bullfrogs in Brazil.

Discussion

Most of the predation records found in this review came from a few studies which assessed the overall dietary composition of *L. catesbeianus*, and revealed that the diet of these invasive frog populations is represented by a wide variety of native anuran species (Boelter and Cechin 2007; Silva et al. 2009, 2010, 2011). Only two predation records of *L. catesbeianus* and native anurans in the field were found, probably due to some difficulty

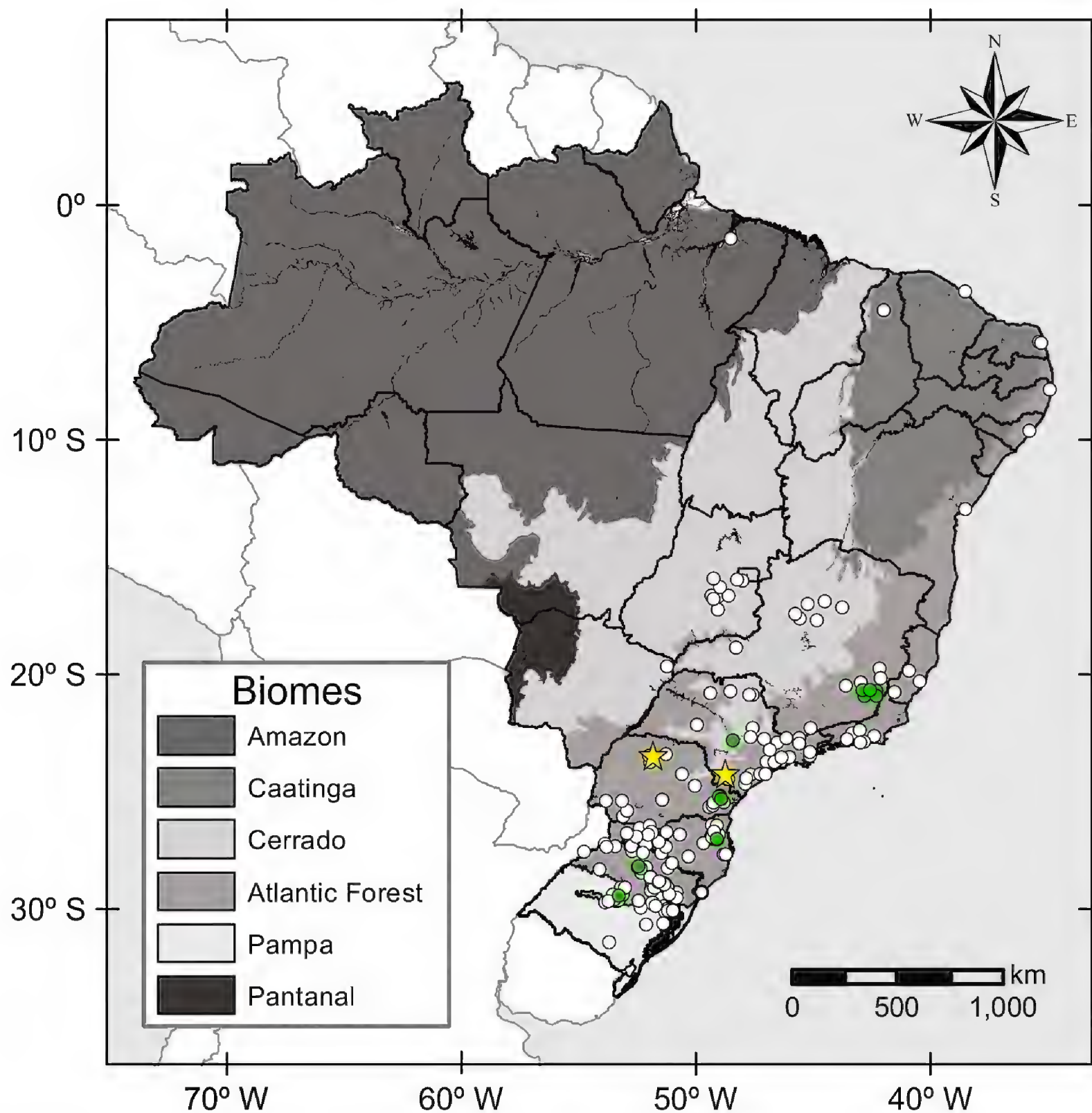


Fig. 3. Spatial distribution of *Lithobates catesbeianus* invasive populations and predation reports of native anurans in Brazil. White circles: American Bullfrog populations in Brazil (Both et al. 2011; Instituto Horus 2016); yellow stars: predation reports of adult *Boana raniceps* and adult *Phyllomedusa distincta* in southern and southeastern Brazil; light green circles: locations of 41 published predation records.

in recording and quantifying these events in the field (Pombal Jr. 2007).

The predicted potential occurrence of *L. catesbeianus* in Brazil represents its current distribution in the southern and southeastern regions in the Atlantic Forest, with potential areas for colonization remaining in the central and northeastern regions (Giovanelli et al. 2008; Both et al. 2011). The results showed that all predation records occurred at sites in southern and southeastern Brazil, regions with higher numbers of research centers, thus contributing a disproportionately greater number of field studies.

Native anurans recorded as prey of American Bullfrogs share the same breeding sites. Silva et al. (2011) had found a spatial overlap in microhabitat use between native species and American Bullfrogs during the reproductive season. American Bullfrogs may also overlap with native amphibians in diet composition

(Bissattini et al. 2019). This may lead to a potential competition, and may have a direct influence on community composition patterns since the intrinsic ecological properties of organisms determine the niche overlap between species in the communities (Vignoli and Luiselli 2012; Vignoli et al. 2017). Additionally, the predation on other anuran species by *L. catesbeianus* can represent an example of intraguild predation (Polis et al. 1989), a process that may facilitate the establishment of the American Bullfrog (Bissattini et al. 2018), as found in other disparate introduced taxa, such as ladybird beetles (Snyder et al. 2004) and fish (Pereira et al. 2015). Intraguild predation can benefit the establishment of *L. catesbeianus* by reducing the competitive pressure by direct predation of the other anuran species.

The number of prey species had a positive correlation with the number of species per genus, in which the family Hylidae had the highest number of species as

Table 1. Native anuran species as prey of *Lithobates catesbeianus* in Brazil based on 41 records obtained from 11 publications, and from two predation events reported here from southern and southeastern Brazil.

Anuran prey species	Body Size ^a (cm)		Locality ^b	Biomes ^c	Reference
	SVL ♂	SVL ♀			
Bufonidae					
<i>Rhinella abei</i> (Baldiissera-Jr, Caramaschi and Haddad, 2004)	6.1	9.3	Quatro Barras, Campina Grande do Sul, and Bocaíúva do Sul, PR	AF	Leivas et al. 2012
<i>Rhinella ornata</i> (Spix, 1824)	6.4	7.9	Viçosa, MG Viçosa, MG	AF, Ce	Reis et al. 2007 Silva et al. 2010
Hylidae					
<i>Dendropsophus elegans</i> (Wied-Neuwied, 1824)	2.9	3.1	Viçosa, MG Viçosa, MG Viçosa, MG	AF, Ca	Silva et al. 2009 Silva et al. 2011 Silva et al. 2016
<i>Dendropsophus minutus</i> (Peters, 1872)	2.3	2.5	Agudo and Nova Palma, RS Agudo and Nova Palma, RS Viçosa, MG Botucatu, SP	AF, Am, Ca, Ce, Pt, Pp	Boelter and Cechin 2007 Boelter et al. 2012 Silva et al. 2009 Almeida 2010
<i>Dendropsophus nanus</i> (Boulenger, 1889)	2.0	2.3	Botucatu, SP	AF, Am, Ca, Ce, Pt, Pp	Almeida 2010
<i>Boana albomarginata</i> (Spix, 1824)	5.1	5.8	Blumenau, SC	AF, Ca, Ce	Dallacorte 2010
<i>Boana bischoffi</i> (Boulenger, 1887)	4.5	5.9	Quatro Barras, Campina Grande do Sul, and Bocaíúva do Sul, PR	AF, Ce, Pp	Leivas et al. 2012
<i>Boana faber</i> (Wied-Neuwied, 1821)	8.8	8.9	Viçosa, MG Blumenau, SC	AF, Ca, ce	Silva et al. 2009 Dallacorte 2010
<i>Boana pulchella</i> (Duméril and Bibron, 1841)	4.2	4.5	Agudo and Nova Palma, RS Agudo and Nova Palma, RS	AF, Pp	Boelter and Cechin 2007 Boelter et al. 2012
<i>Boana raniceps</i> Cope, 1862	7.1	6.8	Maringá, PR	AF, Am, Ca, Ce, Pt	This sudy
<i>Scinax crospedospilus</i> (A. Lutz, 1925)	3.0	3.1	Viçosa, MG	AF	Silva et al. 2009
<i>Scinax eurydice</i> (Bokermann, 1968)	5.3	4.8	Viçosa, MG Agudo and Nova Palma, RS Agudo and Nova Palma, RS	AF, Am, Ca, Ce	Silva et al. 2009 Boelter and Cechin 2007 Boelter et al. 2012
<i>Scinax fuscovarius</i> (A. Lutz, 1925)	4.2	4.5	Botucatu, SP Viçosa, MG Vieiras, MG Vieiras, MG	AF	Almeida 2010 Silva et al. 2010 Silva et al. 2011 Silva et al. 2016

Table 1 (continued). Native anuran species as prey of *Lithobates catesbeianus* in Brazil based on 41 records obtained from 11 publications, and from two predation events reported here from southern and southeastern Brazil.

Anuran prey species	Body Size ^a (cm)		Locality ^b	Biomes ^c	Reference
	SVL ♂	SVL ♀			
Leptodactylidae					
<i>Leptodactylus fuscus</i> (Schneider, 1799)	4.4	4.4	Agudo, and Nova Palma, RS	AF, Am, Ca, Ce, Pp, Pt	Boelter and Cechin 2007
<i>Leptodactylus latrans</i> (Steffen, 1815)	9.6	9.2	Agudo, and Nova Palma, RS	AF, Am, Ca, Ce, Pp, Pt	Boelter et al. 2012
<i>Physalaemus cuvieri</i> Fitzinger, 1826	2.8	3.1	Quatro Barras, Campina Grande do Sul, and Bocaíúva do Sul, PR	AF, Am, Ca, Ce, Pp, Pt	Leivas et al. 2012
<i>Pseudopaludicola mystacalis</i> (Cope, 1887)	1.7	2.1	Agudo and Nova Palma, RS	AF, Am, Ca, Ce, Pp, Pt	Boelter and Cechin 2007
Microhylidae					
<i>Elachistocleis cesarii</i> (Miranda-Ribeiro, 1920)	2.9	3.2	Agudo and Nova Palma, RS	AF, Am, Ca, Ce, Pp, Pt	Boelter et al. 2012
<i>Elachistocleis bicolor</i> (Guérin-Ménéville, 1838)	2.8	3.8	Vieiras, MG	AF, Am, Ca, Ce, Pp, Pt	Boelter et al. 2012
Odontophrynidae					
<i>Odontophrynus americanus</i> (Duméril and Bibron, 1841)	4.5	4.9	Vieiras, MG	AF, Am, Ca, Ce, Pp, Pt	Boelter et al. 2012
Phyllomedusidae					
<i>Phyllomedusa distincta</i> Lutz, 1950	5.6	6.0	Vieiras, MG	AF, Am, Ca, Ce, Pp, Pt	Boelter et al. 2012

^a Values of snout-vent length (SVL) available in Uetanabaro et al. (2008) and Haddad et al. (2013).

^b Brazilian states: MG = Minas Gerais; PR = Paraná; RS = Rio Grande do Sul; SC = Santa Catarina; SP = São Paulo.

^c Occurrence of anuran species within Brazilian biomes: AF = Atlantic Forest; Am = Amazon; Ca = Caatinga; Ce = Cerrado; Pp = Pampa; Pt = Pantanal.

prey of *L. catesbeianus*. Boelter et al. (2012) found that 60% of the prey records corresponded to Hylidae species, suggesting that this group suffers higher predation pressure. At least two non-mutually exclusive hypotheses may explain these patterns by relating family (i.e., richness, abundance) and species traits (i.e., body size) to *L. catesbeianus* predation rates, involving species traits that are often phylogenetically correlated (Martins and Hansen 1997). Firstly, it is possible that Hylidae species are often preyed upon due to their higher species richness in comparison to other families, which is a plausible hypothesis if we assume that predation rates can be proportional to prey abundance or richness (i.e., higher predation rates in higher resource availability conditions; Jacobsen et al. 2014; Madahi et al. 2015). Secondly, Hylidae species may have a higher predation rate because of their smaller size relative to species from other families (e.g., Bufonidae). Predators that feed on whole animals, such as the American Bullfrog, are limited by the prey's body size. Experimental evidence indicates that larger specimens of *L. catesbeianus* feed preferentially on smaller, rather than on large-sized, native anurans (Wang et al. 2007), suggesting a size-based selection of prey species. Partially related to this hypothesis, our results suggest a higher amount of small and medium-sized species as American Bullfrog prey. Therefore, the preference for prey of a certain body size may be proportionally related to the body size of the predators, as observed in previous studies (Quiroga et al. 2015; Silva et al. 2011, 2009; Wang et al. 2007).

This survey found that all anuran species preyed upon by *L. catesbeianus* have large geographic distributions, occurring in various Brazilian states and biomes (Frost 2019; Toledo and Batista 2012). Both et al. (2014) found that American Bullfrog abundance had a positive relationship with communities that consisted of generalist species (e.g., *Physalaemus cuvieri*, *Dendropsophus minutus*), that were anthropogenically adapted and broadly distributed in South America. Native anurans with large geographic ranges (e.g., *Rhinella diptycha*, *Dendropsophus minutus*, *Boana faber*, *B. raniceps*, *Scinax fuscovarius*, and *Physalaemus cuvieri*) have also been found in sympatry with *Lithobates catesbeianus* elsewhere (Affonso et al. 2014).

Conclusions

This study indicated *L. catesbeianus* preys on at least 21 native anuran species in Brazil. Predation is one of the major negative effects of invasive species on native communities. The quality of being a generalist feeder, preying on many anuran species, has benefited the successful colonization, establishment, and permanence of the American Bullfrog in Brazil (and even worldwide; e.g., Li et al. 2011; Monello et al. 2006; Quiroga et al. 2015). Native species of the family Hylidae may be more susceptible to American Bullfrog predation because of

their higher abundance and richness, and/or due to a higher representation of small- to medium-sized species relative to other anuran families. Knowledge on the species most vulnerable to predation by the American Bullfrog can enable better prediction of the negative impacts of such an invasive species on native anuran communities.

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Literature Cited

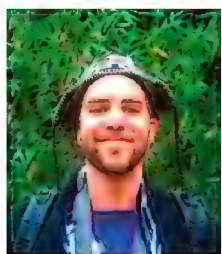
- Almeida SC. 2010. Ecologia de *Lithobates catesbeianus* (Shaw, 1801) e relações com os anfíbios da região de Botucatu, SP (Amphibia, Anura). Ph.D. Dissertation, Universidade Estadual Paulista, Botucatu, SP, Brazil. 79 p.
- Affonso IP, Cafofo EG, Delariva RL, Oda FH, Karling LK, Lourenço-de-Moraes R. 2014. List of anurans (Amphibia: Anura) from the rural zone of the municipality of Maringá, Paraná state, southern Brazil. *Check List* 10(4): 878–882.
- Batista CG. 2002. *Rana catesbeiana* (Bullfrog). Effects on native anuran community. *Herpetological Review* 33(2): 131.
- Batista M, Silva M, Barreto C. 2015. Effects of introduction and decline of a bullfrog population (*Lithobates catesbeianus*) in a community of amphibians in the Cerrado from Central Brazil. *Herpetology Notes* 8: 263–265.
- Bissattini A, Vignoli L. 2017. Let's eat out, there's crayfish for dinner: American Bullfrog niche shifts inside and outside native ranges and the effect of introduced crayfish. *Biological Invasions* 19(9): 2,633–2,646.
- Bissattini A, Buono V, Vignoli L. 2018. Field data and worldwide literature review reveal that alien crayfish mitigate the predation impact of the American Bullfrog on native amphibians. *Aquatic Conservation: Marine and Freshwater Ecosystems* 28(6): 1,465–1,475.

- Bissattini A, Buono V, Vignoli L. 2019. Disentangling the trophic interactions between American Bullfrogs and native anurans: complications due to post-metamorphic ontogenetic niche shifts. *Aquatic Conservation* 29: 270–281.
- Boelter RA, Cechin SZ. 2007. Impact of the bullfrog diet *Lithobates catesbeianus* (Anura, Ranidae) on native fauna: case study from the region of Agudo, RS, Brazil. *Natureza & Conservação* 5(2): 115–123.
- Boelter RA, Kaefer IL, Both C, Cechin S. 2012. Invasive bullfrogs as predators in a Neotropical assemblage: What frog species do they eat? *Animal Biology* 62(4): 397–408.
- Both C, Lingnau R, Santos Jr A, Madalozzo B, Lima LP, Grant T. 2011. Widespread occurrence of the American Bullfrog, *Lithobates catesbeianus* (Shaw, 1802) (Anura: Ranidae), in Brazil. *South American Journal of Herpetology* 6(2): 127–134.
- Both C, Madalozzo B, Lingnau R, Grant T. 2014. Amphibian richness patterns in Atlantic Forest areas invaded by American Bullfrogs. *Austral Ecology* 39(7): 864–874.
- Dallacorte F. 2010. Impacto da Rã-touro-gigante (*Lithobates catesbeianus*) sobre a fauna nativa na zona de amortecimento e interior do Parque Nacional da Serra do Itajaí (PNSI), Blumenau – SC. M.S. Thesis, Universidade Regional de Blumenau, Blumenau, SC, Brazil. 106 p.
- Ficetola GF, Thuller W, Miaud C. 2007. Prediction and validation of the potential global distribution of a problematic alien invasive species – the American Bullfrog. *Diversity and Distributions* 13(4): 476–485.
- Frost DR. 2019. *Amphibian Species of the World: an Online Reference*. Version 6.0. Available: <http://research.amnh.org/herpetology/amphibia/index.html> [Accessed: 12 February 2019].
- Giovanelli JGR, Haddad CFB, Alexandrino J. 2008. Predicting the potential distribution of the alien invasive American Bullfrog (*Lithobates catesbeianus*) in Brazil. *Biological Invasions* 10(5): 585–590.
- Haddad CFB, Toledo LF, Prado CPA, Loebmann D, Gasparini JL, Sazima I. 2013. *Guia dos Anfíbios da Mata Atlântica – Diversidade e Biologia*. Anolis Books, São Paulo, Brazil. 544 p.
- Instituto Hórus. 2016. Base de dados de espécies exóticas invasoras no Brasil. Instituto Hórus, Florianópolis, SC, Brazil. Available: <http://www.institutohorus.org.br> [Accessed: 20 October 2016].
- IUCN. 2012. *Biological Invasions: a Growing Threat to Biodiversity, Human Health and Food Security. Policy Recommendation for the Rio+20 Process Drafted by IUCN Species Survival Commission Invasive Species Specialist Group and Invasive Species Initiative*. International Union for Conservation of Nature (IUCN), Gland, Switzerland. Available: http://cmsdata.iucn.org/downloads/policy_brief_on_invasive_and_alien_species_3.pdf/ [Accessed: 13 October 2016].
- Jacobsen L, Berg S, Baktoft H, Nilsson PA, Skov C. 2014. The effect of turbidity and prey fish density on consumption rates of piscivorous Eurasian perch *Perca fluviatilis*. *Journal of Limnology* 73(1): 187–190.
- Kiesecker JM, Blaustein AR. 1998. Effects of introduced bullfrogs and small-mouth bass on the microhabitat use, growth, and survival of native red-legged frogs. *Conservation Biology* 12(4): 776–787.
- Kraus F. 2009. *Alien Reptiles and Amphibians: a Scientific Compendium and Analysis*. Springer, New York, New York, USA. 563 p.
- Leivas PT, Leivas FWT, Moura MO. 2012. Diet and trophic niche of *Lithobates catesbeianus* (Amphibia: Anura). *Zoologia* 29(5): 405–412.
- Leivas PT, Savaris M, Lampert S, Lucas EM. 2013. Predation of *Odontophrynus americanus* (Anura: Odontophrynidae) by the invasive species *Lithobates catesbeianus* (Anura: Ranidae) in an Araucaria Forest remnant in Southern Brazil. *Herpetology Notes* 6: 603–606.
- Li YM, Ke ZW, Wang YH, Blackburn TM. 2011. Frog community responses to recent American Bullfrog invasions. *Current Zoology* 57(1): 83–92.
- Lima SL, Agostinho CA. 1988. *A Criação de Rãs*. Editora Globo, Rio de Janeiro, Brazil. 187 p.
- Liu X, Wang S, Ke Z, Cheng C, Wang Y, Zhang F, Xu F, Li X, Gao X, Jin C, et al. 2018. The invaders do not result in heavier impacts: the effects of non-native bullfrogs on native anurans are mitigated by high densities of non-native crayfish. *Journal of Animal Ecology* 87(3): 850–862.
- Lowe S, Browne M, Boudjelas S, De Poorter M. 2004. *100 of the World's Worst Invasive Alien Species: a Selection from the Global Invasive Species Database*. The Invasive Species Specialist Group (ISSG), a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), Gland, Switzerland. Available: http://www.issg.org/database/species/reference_files/100english.pdf/ [Accessed: 13 October 2016].
- Loyola RD, Nabout JC, Trindade-Filho J, Lemes P, Urbina-Cardona JN, Dobrovolski R, Sagnori MD, Diniz-Filho JAF. 2012. Climate change might drive species into reserves: a case study of the American Bullfrog in the Atlantic Forest biodiversity hotspot. *Alytes* 29(1-4): 61–74.
- Madahi K, Sahragard A, Hosseini R. 2015. Predation rate and numerical response of *Aphidoletes aphidimyza* feeding on different densities of *Aphis craccivora*. *Biocontrol Science and Technology* 25(1): 72–83.
- Martins EP, Hansen TF. 1997. Phylogenies and the comparative method: A general approach to incorporating phylogenetic information into the analysis of interspecific data. *American Naturalist* 149(4): 646–667.

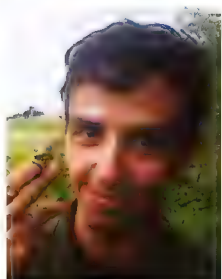
- Monello RJ, Dennehy JJ, Murray DL, Wirsing AJ. 2006. Growth and behavioral responses of tadpoles of two native frogs to an exotic competitor, *Rana catesbeiana*. *Journal of Herpetology* 40(3): 403–407.
- Nori J, Urbina-Cardona JN, Loyola RD, Lescano JN, Leynaud GC. 2011. Climate change and American Bullfrog invasion: What could we expect in South America? *PLoS ONE* 60: e25718.
- Pereira LS, Agostinho AA, Gomes LC. 2015. Eating the competitor: a mechanism of invasion. *Hydrobiologia* 746(1): 223–231.
- Polis GA, Myers CA, Holt RD. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annual Review of Ecology, Evolution, and Systematics* 20: 297–330.
- Pombal Jr JP. 2007. Notas sobre predação em uma taxocenose de anfíbios anuros no sudeste do Brasil. *Revista Brasileira de Zoologia* 24(3): 841–843.
- Quiroga L, Moreno MD, Cataldo AA, Aragón-Traverso JH, Pantano MV, Olivares JPS, Sanabria EA. 2015. Diet composition of an invasive population of *Lithobates catesbeianus* (American Bullfrog) from Argentina. *Journal of Natural History* 49(24–28): 1,703–1,716.
- Reis EP, Silva ET, Feio RN, Filho OPR. 2007. *Chaunus pombali* (Pombali's toad). Predation. *Herpetological Review* 38(3): 321.
- Silva ET, Both C, Filho OPR. 2016. Food habits of invasive bullfrogs and native thin-toed frogs occurring in sympatry in southeastern Brazil. *South American Journal of Herpetology* 11(1): 25–33.
- Silva ET, Ribeiro-Filho OP, Feio RN. 2011. Predation of native anurans by invasive bullfrogs in southeastern Brazil: spatial variation and effect of microhabitat use by prey. *South American Journal of Herpetology* 6(1): 1–10.
- Silva ET, Reis EP, Feio RE, Filho OPR. 2009. Diet of the invasive frog *Lithobates catesbeianus* (Shaw, 1802) (Anura: Ranidae) in Viçosa, Minas Gerais state, Brazil. *South American Journal of Herpetology* 4(3): 286–294.
- Silva ET, Reis EP, Santos PS, Feio RN. 2010. *Lithobates catesbeianus* (American Bullfrog). Diet. *Herpetological Review* 41(4): 475–476.
- Snyder WE, Clevenger GM, Eigenbrode SD. 2004. Intraguild predation and successful invasion by introduced ladybird beetles. *Oecologia* 140(4): 559–565.
- Toledo LF, Batista RF. 2012. Integrative study of Brazilian anurans: geographic distribution, size, environment, taxonomy, and conservation. *Biotropica* 44(6): 785–792.
- Toledo LF, Ribeiro RS, Haddad CFB. 2007. Anurans as prey: an exploratory analysis and size relationships between predators and their prey. *Journal of Zoology* 271(2): 170–177.
- Uetanabaro M, Prado CPA, Rodrigues DJ, Gordo M, Campos Z. 2008. *Guia de Campo dos Anuros do Pantanal Sul e Planaltos de Entorno*. Editora UFMS/UFMT, Campo Grande, MS, Brazil. 196 p.
- Vizotto LD. 1984. Ranicultura. *Ciência e Cultura* 36(1): 42–45.
- Wang YP, Guo ZW, Pearl CA, Li YM. 2007. Body size affects the predatory interactions between introduced American Bullfrogs (*Rana catesbeiana*) and native anurans in China: an experimental study. *Journal of Herpetology* 41(3): 514–520.



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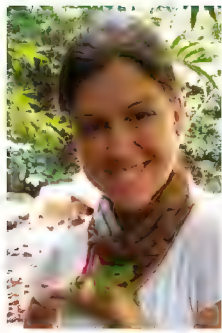
Impact of bullfrogs on native anurans in Brazil



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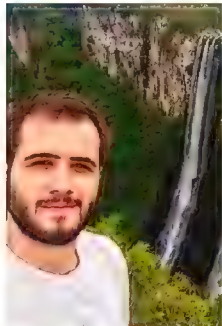
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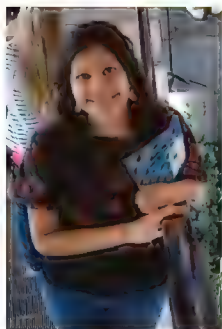
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Biology of snakes of the genus *Tretanorhinus*: an integrative review

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Abstract.—Many aspects of the biology of various snake species remain unknown, and the extent of this lack of information is not always clear. As new research usually depends upon previous findings, the gaps in our knowledge and the accuracy of published information are of major importance. Therefore, an analysis of all available information on snakes of the genus *Tretanorhinus*, from both the literature and museum specimens, is presented here to illuminate existing knowledge gaps. The database compiled from 87 documents referring to snakes of this genus and 755 specimens held in scientific collections revealed major gaps and contradictory information for all four species of this genus. Data on morphology, ecology, and natural history are completely absent for *T. mocquardi* and *T. taeniatus*, whereas confusing distribution reports exist for *T. nigroluteus*. The potential consequences of these problems were determined, and some suggestions for correcting them are addressed. Specifically, we consider that focused efforts on the validation of current species and subspecies, field and lab studies of ecology and behavior, and estimations of population dynamics, are necessary.

Keywords. Colubridae, Dipsadidae, ecology, literature review, morphology, museum specimens, natural history, Reptilia, Serpentes, taxonomy

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Introduction

Fieldwork with snakes usually poses several challenges and more attention has focused on snake species from temperate areas than those from the tropics (Avila et al. 2006), despite the latter having higher diversity (Greene 1997). This bias has resulted in a lack of key information on essential aspects of the ecology, natural history, and behavior of many species. The collection of such data is a time-consuming and difficult task, as many snake species have cryptic habits and occur in low densities (Greene 1997). However, the lack of basic information for many snake species is a major concern, because this information is crucial for making general interspecific comparisons, establishing proper phylogenetic relationships, determining population trends and dynamics, and recognizing differences in behavior. Therefore, reviewing the current state of knowledge for a given species is imperative to identify the areas that require more attention and to guide research and conservation efforts in the proper direction.

Snakes of the genus *Tretanorhinus* are nocturnal, aquatic species that have intrigued biologists for more than a century (Cope 1861; Duméril et al. 1854). Their

habits and secretive behavior make them difficult to study, so their biology remains largely unknown (Savage 2002; Schwartz and Henderson 1991). Currently, four species are recognized in the genus: *T. mocquardi* (Bocourt 1891), *T. nigroluteus* (Cope 1861), *T. taeniatus* (Boulenger 1903), and *T. variabilis* (Duméril et al. 1854). Although a handful of studies have increased our knowledge about these species (e.g., Barquero et al. 2005; Dunn 1939; Henderson and Hoever 1979; Villa 1970), the genus remains poorly studied.

Here we summarize and integrate all available published information, highlight the gaps in our knowledge of the biology of *Tretanorhinus* species, and make suggestions to direct future research in order to fill in these gaps. To achieve this, searches were conducted to find all published material referring directly (i.e., studies focused specifically on one or more *Tretanorhinus* species) or indirectly (i.e., studies focused on many taxa that mentioned one or more *Tretanorhinus* species as part of the topic) to snakes of this genus and a database of key information was compiled (Appendix 1). The information mentioned in each study was used to determine missing data for each species. Each publication was classified using the following categories:

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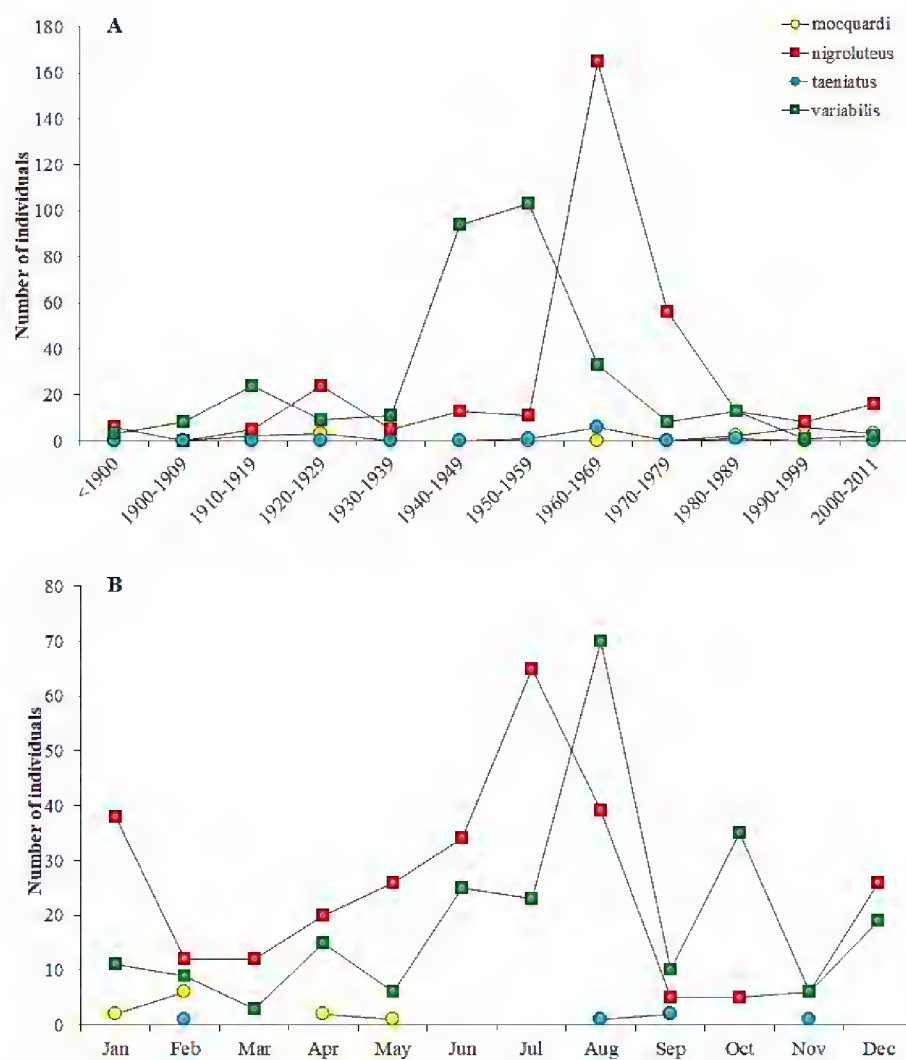


Fig. 1. Number of specimens of the four species of *Tretanorhinus* according to (A) decade and (B) month of collection.

natural history, morphology, taxonomy, systematics, ecology, distribution, biogeography, and reproduction. In addition, data were obtained for specimens held in scientific collections (Appendix 2) from the HerpNet2 data portal (<http://www.herpnet.org>), Global Biodiversity Information Facility (<http://www.gbif.org>), Data Research Warehouse Information Network (<http://darwin.naturalsciences.be>), and by directly contacting collection curators. When available, the following data were extracted: taxonomic classification, type status, sex and age classes, country and locality of the point of capture, latitude and longitude of the collection site, date of collection, and remarks about the individual collected.

Literature Review and Scientific Collections

The search for publications referring to *Tretanorhinus* produced a total of 87 documents, although only 16 focused directly on species of the genus (Appendix 1). Most of the documents focused on general aspects of natural history (31%), taxonomy (21.8%), and biogeography (20.7%), and the vast majority referred to *T. nigroluteus* or *T. variabilis*, with only a handful of studies mentioning *T. mocquardi* ($n = 12$) or *T. taeniatus* ($n = 8$).

A database with information for a total of 755 specimens of *Tretanorhinus* held in 31 scientific collections was compiled (Appendix 2). Most records corresponded to *T. nigroluteus* ($n = 350$) or *T. variabilis* ($n = 357$), and only a few were available for *T. mocquardi* ($n = 25$) or *T. taeniatus* ($n = 9$). In addition, eight records

were identified only to the genus level, and six were mistakenly identified as *Tretanorhinus agassizi*. Most of the collections occurred between the 1940s and 1970s (Fig. 1A) and seasonally between June and August (Fig. 1B), although many records did not include either the year or month of collection.

Distribution and Habitat

Tretanorhinus is a genus exclusive to Middle and South America, and the West Indies. *Tretanorhinus variabilis* is the only one of the species with an unambiguous distribution, inhabiting Cuba, Isla de la Juventud (Isle of Youth), and the Cayman Islands (Fig. 2). *Tretanorhinus nigroluteus* has been frequently recorded along the Atlantic coast from southern Mexico to Colombia, including some islands such as the Bay Islands of Honduras and Great Corn Island of Nicaragua (Fig. 2). However, records of this species in Colombia are controversial. Alarcón-Pardo (1978) reported finding *T. nigroluteus* on the Atlantic coast of Colombia, but we were unable to locate any specimens of this species in the scientific collections consulted which matched the locality mentioned by that author. We found only one specimen of *T. nigroluteus* from the Pacific coast of Colombia as a disjunct point of the species range (Table 1). The distributions of *T. mocquardi* and *T. taeniatus* are the least clear among the species of the genus. *Tretanorhinus mocquardi* ranges from the Canal Zone in Panama through the Pacific coast of Colombia and Ecuador, although it has been reported from only two locations in Colombia and one in Ecuador (Fig. 2, Table 1). *Tretanorhinus taeniatus* could be endemic to Ecuador (Table 1), despite previous reports of this species in Colombia (Castaño-M. et al. 2004; Daniel 1949). Based on these distributions, *T. mocquardi* and *T. taeniatus* could be sympatric in Esmeraldas province, northwestern Ecuador, whereas *T. mocquardi* and *T. nigroluteus* seem to be sympatric in Chocó department on the Pacific coast of Colombia (Fig. 2).

Most information on the preferred habitat of the genus comes from studies conducted on *T. nigroluteus* and *T. variabilis*. *Tretanorhinus* is a fully aquatic genus, inhabiting all kinds of fresh and brackish water bodies such as rivers, streams, lagoons, estuaries, mangroves (Cisneros-Heredia 2005; Neill 1958; Villa 1970), and even cow wells (Seidel and Franz 1994). The species require a muddy or rocky bottom with aquatic vegetation where they can hide and rest (Neill 1965; Villa 1970). Although there are reports of individuals found out of the water (e.g., crossing roads), these events occur after flooding that forces the snakes to search for another water body (Barquero et al. 2005; Villa 1970). The distributions of *T. nigroluteus* and *T. variabilis* also confirm the ability of these snakes to disperse and survive in salt water (Barbour and Amaral 1924; Neill 1958), since they have colonized several islands. In that regard, we observed

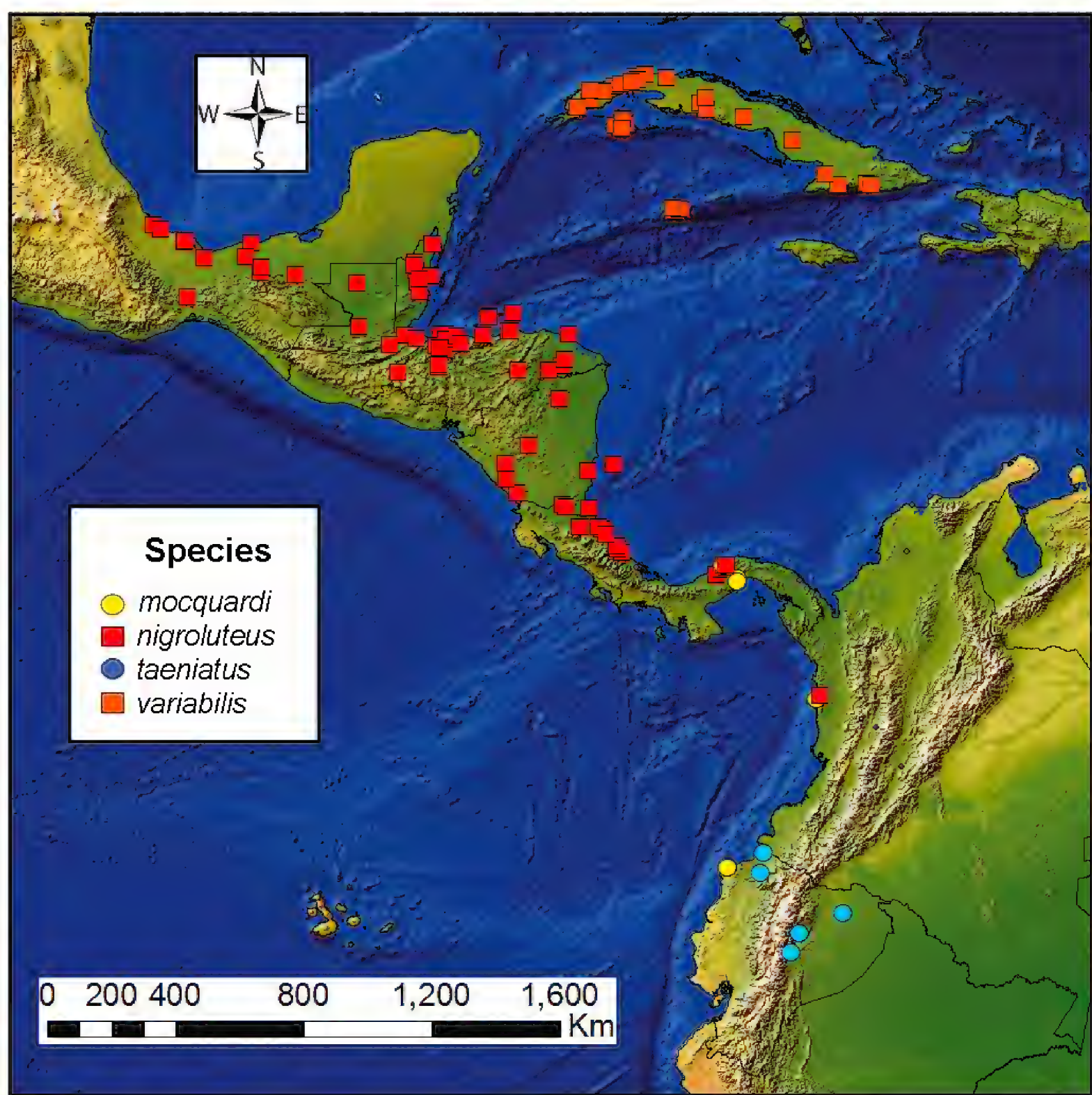


Fig. 2. Map showing the current distribution of the four species of *Tretanorhinus* based on specimens from scientific collections.

Table 1. Number of specimens of *Tretanorhinus* species collected from different countries and held in several scientific collections. Specimens with a doubtful or missing location were excluded (n = 38).

Country	<i>T. mocquardi</i>	<i>T. nigroluteus</i>	<i>T. taeniatus</i>	<i>T. variabilis</i>	Total
West Indies					
Cayman Islands	-	-	-	58	58
Cuba	-	-	-	280	280
Middle America					
Belize	-	52	-	-	52
Costa Rica	-	11	-	-	11
Guatemala	-	11	-	-	11
Honduras	-	157	-	-	157
Mexico	-	30	-	-	30
Nicaragua	-	65	-	-	65
Panama	14	19	-	-	33
South America					
Colombia	4	1	-	-	5
Ecuador	6	-	9	-	15
Total	24	346	9	338	717

Table 2. Morphological information available in the literature for each species of *Tretanorhinus*.

Trait	<i>T. mocquardi</i>	<i>T. nigroluteus</i>	<i>T. taeniatus</i>	<i>T. variabilis</i>
SVL (mm)				
Adults	-	242–656	440	500–800
Juveniles	-	140–168	-	143–145
Tail length (mm)				
Adults	-	142–206	130	145–160
Juveniles	-	51–77	-	-
No. of loreals	1	1–2	1	1–3
No. of prefrontals	1–2	2	3	2
No. of preoculars	2	2–3	2	1–3
No. of postoculars	-	2	2	2
No. of temporals	-	1+2	1+2, 2+3	1+2
No. of upper labials	-	7–9	8	8–9
No. of lower labials	-	9–12	4–5	9–11
No. of ventrals	166–177	127–151	168–175	152–168
No. of caudals	69–85	56–81	74–81	48–81
Posterior chin-shields	In contact	In contact	Separated	Separated
No. of dorsal rows	19	19, 21	21	19, 21
Ventral color	Fuliginous yellow	Orange, light red, yellow, cream	-	Bluish gray, dark brown
Ventral pattern	-	With or without dots or spots	3 dark stripes	Light dots or spots
Dorsal color	-	Olive, grayish brown, light brown, black	Grayish olive	Grayish olive, dark brown
Dorsal pattern	3 longitudinal stripes	With or without dark spots	3 longitudinal stripes	Blackish cross bands

an individual of *T. nigroluteus* resting on a beach on the Caribbean coast of Costa Rica after a heavy rain, suggesting that the snake had been washed out to sea, surviving until it was returned to land by the tide.

Morphology

Morphological information is limited and incomplete for *T. mocquardi* and *T. taeniatus*, despite both species being described more than 110 yrs ago by Bocourt (1891) and Boulenger (1903), respectively. Table 2 provides a summary of some morphological features that were extracted from the literature review. Overall, *Tretanorhinus* are relatively small snakes; *T. variabilis* is the largest species with an SVL of up to 800 mm. All four species in the genus exhibit a grayish dorsal coloration with stripes, spots, or bands, and a yellow, orange, or gray ventral coloration. Juveniles have been found in the wild only infrequently, and the sex of most collected individuals was not identified (Table 3). Juveniles of each species resemble the adults in pattern and coloration (Barquero et al. 2005; Petzold 1967).

Previous attempts to produce a key to the species of the genus have never included all four species. Therefore, the following key is presented to unify the previous efforts (Bocourt 1891; Boulenger 1893; Dunn 1939; Peters and Orejas-Miranda 1970; Köhler 2008) and incorporate more recent data.

Key to the species of *Tretanorhinus*:

- 1a. Dorsum without stripes, more than one loreal can be present.....2
- 1b. Dorsum with three longitudinal stripes, only one loreal 3
- 2a. Posterior chin-shields in contact, ventrals fewer than 152.....*nigroluteus*
- 2b. Posterior chin-shields separated, ventrals 152 or more.....*variabilis*
- 3a. Less than three prefrontals, posterior chin-shields in contact.....*mocquardi*
- 3b. Three prefrontals, posterior chin-shields separated*taeniatus*

Taxonomy and Systematics

The evolutionary relationships of the genus are enigmatic and controversial, so that *Tretanorhinus* has been placed within different former subfamilies of Colubridae (e.g., Natricinae, Xenodontinae, and Dipsadinae) by various authors (Crother 1999; Dowling et al. 1996; Pinou and Dowling 1994; Villa 1969). The general consensus is that the genus should be placed within Dipsadidae (formerly Dipsadinae, Grazziotin et al. 2012; Pinou and Dowling 1994) and it is commonly referred to as a xenodontine (Cadle 1985; Minton 1976; Vidal et al. 2000). Moreover, the phylogenetic relationships with other genera are still ambiguous. Crother (1999) placed *Tretanorhinus* as the sister taxon of *Sibon* or the *Sibon-Manolepis* clade. In two studies that included more taxa, Grazziotin et al. (2012) noted that *Tretanorhinus* could be the sister taxon of *Hypsiglena*, and both genera were placed in a clade containing *Trimetopon*, *Geophis*, and *Atractus*; whereas Pyron et al. (2013) placed *Tretanorhinus* as the sister taxon of the *Leptodeira-Imantodes* clade. However, in all these studies the relationships of *Tretanorhinus* with other taxa were poorly supported and the genus is considered as Dipsadidae *incertae sedis* (Grazziotin et al. 2012).

The affinities of each species within *Tretanorhinus* are also enigmatic, and this is caused by the lack of information on the basic biological aspects of some species. Detailed morphological information is only available for *T. nigroluteus* and *T. variabilis* (Table 2). No author has given a complete morphological description of *T. mocquardi*, such that even basic phenotypic traits (e.g., most head scutellation and dorsal coloration) are missing (Table 2). In the case of *T. taeniatus*, morphological information has only been reported for females, as males have never been deposited or identified as such in scientific collections (Table 3). Despite these deficiencies, morphological information compiled from the literature review here suggests that *T. taeniatus* and *T. mocquardi* are more closely related to each other than to *T. nigroluteus* and *T. variabilis*.

Problems have also arisen with the subspecies described for both *T. nigroluteus* (*dichromaticus*, *lateralis*, *mertensi*, *nigroluteus*, and *obscurus*) and *T. variabilis* (*binghami*, *insulaepinorum*, *lewisi*, *variabilis*, and *wagleri*). The morphological variables used to define the subspecies have been chosen arbitrarily (Wilson and Hahn 1973). For example, differences in coloration have been used by some authors to differentiate among subspecies with only superficial descriptions (Schwartz and Ogren 1956; Smith 1965; Villa 1969). In addition, no genetic analyses have yet been reported to confirm the validity of these subspecies.

Ecology and Natural History

Information on the ecology and natural history of *T. mocquardi* and *T. taeniatus* is virtually non-existent

Table 3. Number of specimens of *Tretanorhinus* held in scientific collections that have been identified as female, male, or juvenile.

Species	Female	Male	Juvenile	Total
<i>T. mocquardi</i>	2	1	-	3
<i>T. nigroluteus</i>	14	16	2	32
<i>T. taeniatus</i>	2	-	-	2
<i>T. variabilis</i>	19	17	4	40
Total	37	34	6	77

and they remain poorly studied for *T. nigroluteus* and *T. variabilis*. Snakes of *Tretanorhinus* are nocturnal and seem to hide during the day in water bodies amongst roots and rock crevices (Barbour and Ramsden 1919; Stuart 1937; Villa 1970). Some unusual features have been identified for the genus. For example, individuals of *Tretanorhinus* feed upon fishes, tadpoles, and frogs by either actively chasing prey or remaining motionless with the tail and body attached to a supporting surface (e.g., branch or rock) and striking at passing prey (Barquero et al. 2005; Neill 1965; Petzold 1967; Wilson and Hahn 1973). In addition, these snakes demonstrate shy behavior, such as fleeing to the bottom of water bodies when disturbed (Stuart 1937) and rolling up the body like a ball when caught (Petzold 1967; Seidel and Franz 1994). Known natural predators of *Tretanorhinus* include, but are probably not limited to, turtles (e.g., *Kinosternon* [Villa 1973]) and wading birds (e.g., *Tigrissoma* and *Cochlearius* [Villa 1970]). A specimen from Costa Rica was collected from a crab, thus confirming the assumption of Villa (1970) that some species of crabs can be predators of *Tretanorhinus*.

Information on reproduction is scarce for all four species of *Tretanorhinus*, although observations in captivity demonstrate that *T. nigroluteus* and *T. variabilis* are oviparous, laying 6–9 adherent eggs out of water (Barquero et al. 2005; Petzold 1967). *Tretanorhinus variabilis* lays larger eggs (35[L] x 16.75[W] mm on average) that hatch earlier (35 d) than *T. nigroluteus* (21.5[L] x 10[W] mm, 42 d). Villa (1970) found gravid females of *T. nigroluteus* during both the dry and wet seasons, suggesting that reproduction could occur year-round in this species. Most gravid females of *T. variabilis* have been found during the wet season in July and August (Petzold 1967; Seidel and Franz 1994). Both *T. nigroluteus* and *T. variabilis* are sexually dimorphic, with females being larger than males and only males possessing tubercles on scales of the head (Henderson and Hoevers 1979; Petzold 1967).

The capacity to survive in salt water likely contributed to the colonization of Caribbean islands and northeastern South America from a Central American ancestor (Cisneros-Heredia 2005; Hedges 1996) and allowed *T. nigroluteus* and *T. variabilis* to become fairly abundant in some parts of their ranges (Henderson and Hoevers 1977; Schwartz and Henderson 1991). Henderson and Hoevers (1977) reported that *T. nigroluteus* was more frequently

found during the dry season (December to May) than during the wet season (June to November) of Belize, a difference explained by the overflowing of the river system studied. However, historical collections of this species show that more individuals have been collected during the wet season, a pattern shared with *T. variabilis* (Fig. 1), and in accordance with reports by Wilson and Hahn (1973) for Roatan Island, Honduras. *Tretanorhinus mocquardi* and *T. taeniatus* are less abundant than the other two species and no pattern of variation in abundance can be elucidated from available data.

General Considerations and Future Research

This study has summarized information about the species of the genus *Tretanorhinus* published from 1854 (Duméril et al. 1854) to the present (Estrella-Morales and Piedra-Castro 2018). Information was also incorporated on all collected specimens of this genus that could be identified as preserved in scientific collections throughout the world. This integrative approach allowed the identification of gaps in our knowledge about these snakes. For example, it is surprising that (1) a complete morphological description is not available in the literature for *T. mocquardi*, (2) only a few specimens have been collected for *T. mocquardi* and *T. taeniatus*, and (3) most natural history and ecological information simply has never been reported for any of the species. In addition to the lack of key data, much available information is contradictory, such as the reported occurrence of *T. nigroluteus* and *T. taeniatus* in Colombia, which can cause several problems. Therefore, one can ask how a reliable identification of individuals in the field can be made when such basic data are missing. This is particularly problematic for sympatric species, such as *T. nigroluteus* and *T. mocquardi* in Panama, and for *T. mocquardi* and *T. taeniatus* in Ecuador.

In order to fill in the gaps in our knowledge of *Tretanorhinus*, future research should focus on at least three areas. First, validation of the currently accepted species and subspecies is absolutely urgent. Barbour and Amaral (1924) have questioned the validity of *T. mocquardi* (although see Dunn 1939), while Wilson and Hahn (1973) refused to recognize *T. n. dichromaticus*. Therefore, a comparative study of all species and subspecies that includes both morphological and genetic data and produces a phylogeny of the genus is necessary. Previous attempts have failed to include all species or have used only morphological or genetic data. Second, both field and lab studies are needed to increase our knowledge about these secretive and, in some areas, elusive species. *Tretanorhinus mocquardi* and *T. taeniatus* require extensive work on morphological variation, ecological habits, distribution patterns, and natural history traits. Breeding behavior is completely unknown for these two species and males of *T. taeniatus* have yet to be measured and described. Although

there is significantly more information available for *T. nigroluteus* and *T. variabilis*, nothing is known about their courtship, sexual selection, development, and many other aspects of basic biology. Third, demographic variation and population dynamics need to be quantified to understand the movement of individuals among populations, sex ratios, and population sizes. These types of data are essential for determining the conservation status of species. Some efforts have already been made to identify areas of high and low abundance across the ranges of *T. nigroluteus* and *T. variabilis*. However, long-term studies which monitor changes in populations are yet to be done.

The problems mentioned above are not restricted only to *Tretanorhinus* species, as they also apply to many other snakes (Dorcas and Willson 2009), and it is alarming that we rely so heavily on unconfirmed or erroneous information. Snakes in particular require special attention due to the intrinsic difficulties in generating accurate information. These difficulties arise from certain aspects of their biology, including low densities, great mobility, and cryptic habits. Integrative studies, such as this one, are important for identifying the gaps in our knowledge of different taxa and guiding future efforts in the right direction.

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Literature Cited

- Alarcón-Pardo H. 1978. Primer registro de *Tretanorhinus nigroluteus nigroluteus* Cope (Reptilia: Serpentes: Colubridae) para Colombia. *Lozania* 27: 1–4.
- Avila RW, Ferreira VL, Arruda JAO. 2006. Natural history of the South American water snake *Helicops leopardinus* (Colubridae: Hydropsini) in the Pantanal, Central Brazil. *Journal of Herpetology* 40: 274–279.
- Barbour T, Amaral AD. 1924. Notes on some Central American snakes. *Occasional Papers of the Boston Society of Natural History* 5: 129–132.
- Barbour T, Ramsden CT. 1919. The herpetology of Cuba. *Memoirs of the Museum of Comparative Zoölogy at Harvard College* 47: 69–213.
- Barquero MD, Campos-Chinchilla P, Valverde R. 2005. *Tretanorhinus nigroluteus*: biology and captive maintenance of the Orangebelly Swamp Snake. *Reptilia* 42: 47–52.
- Bocourt MF. 1891. Sur quelques ophidiens de l’Amérique intertropicale: appartenant au genre *Tretanorhinus*. *Le Naturaliste* 101: 121–122.
- Boulenger GA. 1893. *Catalogue of the Snakes in the British Museum (Natural History)*. Volume I. Taylor and Francis, London, United Kingdom. 504 p.

- Boulenger GA. 1903. Descriptions of new snakes in the collection of the British Museum. *Annals and Magazine of Natural History; Zoology, Botany, and Geology* Series 7, 12: 350–354.
- Cadle JE. 1985. The Neotropical colubrid snake fauna (Serpentes: Colubridae): lineage components and biogeography. *Systematic Zoology* 34: 1–20.
- Castaño-M OV, Cárdenas-A G, Hernández-R EJ, Castro-H F. 2004. Reptiles en el Chocó biogeográfico. Pp. 599–632 In: *Diversidad Biótica IV. El Chocó Biogeográfico/Costa Pacífica*. Editor, Rangel-Ch JO. Universidad Nacional de Colombia, Instituto de Ciencias Naturales, Conservation International, Bogotá, Colombia. 997 p.
- Cisneros-Heredia DF. 2005. *Tretanorhinus mocquardi* (Swamp Snake). *Herpetological Review* 36: 340.
- Cope ED. 1861. Contributions to the ophiology of Lower California, Mexico, and Central America. *Proceedings of the Academy of Natural Sciences of Philadelphia* 13: 292–306.
- Crother BI. 1999. Phylogenetic relationships among West Indian xenodontine snakes (Serpentes; Colubridae) with comments on the phylogeny of some mainland xenodontines. *Contemporary Herpetology* 2: 7–30.
- Daniel H. 1949. Las serpientes de Colombia. *Revista Facultad Nacional de Agronomía Medellín* 10: 301–333.
- Dorcas ME, Willson JD. 2009. Innovative methods for studies of snake ecology and conservation. Pp. 5–37 In: *Snakes: Ecology and Conservation*. Editors, Mullin SJ, Seigel RA. Cornell University Press, Ithaca, New York, USA. 365 p.
- Dowling HG, Hass CA, Hedges SB, Highton R. 1996. Snake relationships revealed by slow-evolving proteins: a preliminary survey. *Journal of Zoology* 240: 1–28.
- Duméril AMC, Bibron G, Duméril A. 1854. *Erpétologie Générale ou Histoire Naturelle Complète des Reptiles*. Volume 7 (partie 1). Librairie Encyclopédique de Roret, Paris, France. 780 p.
- Dunn ER. 1939. Mainland forms of the snake genus *Tretanorhinus*. *Copeia* 1939: 212–217.
- Estrella-Morales J, Piedra-Castro L. 2018. Anfíbios y reptiles (herpetofauna) en las asociaciones vegetales de la Laguna de Gandoca, Limón, Costa Rica. *Tecnología en Marcha* 31: 127–135.
- Greene HW. 1997. *Snakes: the Evolution of Mystery in Nature*. University of California Press, Berkeley, California, USA. 365 p.
- Grazziotin FG, Zaher H, Murphy RW, Scrocchi G, Benavides MA, Zhang YP, Bonatto SL. 2012. Molecular phylogeny of the New World Dipsadidae (Serpentes: Colubroidea): a reappraisal. *Cladistics* 28: 437–459.
- Hedges SB. 1996. The origin of West Indian amphibians and reptiles. Pp. 95–128 In: *Contributions to West Indian Herpetology: a Tribute to Albert Schwartz*. Editors, Powell R, Henderson RW. Society for the Study of Amphibians and Reptiles, Ithaca, New York, USA. 457 p.
- Henderson RW, Hoevers LG. 1977. The seasonal incidence of snakes at a locality in northern Belize. *Copeia* 1977: 349–355.
- Henderson RW, Hoevers LG. 1979. Variation in the snake *Tretanorhinus nigroluteus lateralis* in Belize with notes on breeding tubercles. *Herpetologica* 35: 245–248.
- Köhler G. 2008. *Reptiles of Central America*. 2nd Edition. Herpeton-Verlag, Offenbach, Germany. 400 p.
- Minton SA Jr. 1976. Serological relationships among some congeneric North American and Eurasian colubrid snakes. *Copeia* 1976: 672–678.
- Neill WT. 1958. The occurrence of amphibians and reptiles in saltwater areas, and a bibliography. *Bulletin of Marine Science of the Gulf and Caribbean* 8: 1–97.
- Neill WT. 1965. Notes on aquatic snakes, *Natrix* and *Tretanorhinus*, in Cuba. *Herpetologica* 21: 172–181.
- Peters JA, Orejas-Miranda B. 1970. Catalogue of the Neotropical Squamata. Part I. Snakes. *Bulletin United States National Museum* 297: 1–347.
- Petzold HG. 1967. Some remarks on the breeding biology and the keeping of *Tretanorhinus variabilis*, a water snake of Cuba. *Herpetologica* 23: 242–246.
- Pinou T, Dowling HG. 1994. The phylogenetic relationships of the Central American snake *Tretanorhinus*: data from morphology and karyology. *Amphibia-Reptilia* 15: 297–305.
- Pyron RA, Burbrink FT, Wiens JJ. 2013. A phylogeny and revised classification of Squamata, including 4,161 species of lizards and snakes. *BMC Evolutionary Biology* 13: 93.
- Savage JM. 2002. *The Amphibians and Reptiles of Costa Rica: a Herpetofauna between Two Continents, between Two Seas*. University of Chicago Press, Chicago, Illinois, USA. 954 p.
- Schwartz A, Henderson RW. 1991. *Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History*. University of Florida Press, Gainesville, Florida, USA. 720 p.
- Schwartz A, Ogren LH. 1956. A collection of reptiles and amphibians from Cuba, with the descriptions of two new forms. *Herpetologica* 12: 91–110.
- Seidel ME, Franz R. 1994. Amphibians and reptiles (exclusive of marine turtles) of the Cayman Islands. Pp. 407–433 In: *The Cayman Islands: Natural History and Biogeography*. Editors, Brunt MA, Davies JE. Kluwer Academic Publishers, Dordrecht, Netherlands. 604 p.
- Smith HM. 1965. Two new colubrid snakes from the United States and Mexico. *Journal of the Ohio Herpetological Society* 5: 1–4.
- Stuart LC. 1937. Some further notes on the amphibians and reptiles of the Peten forest of northern Guatemala. *Copeia* 1937: 67–70.

Biology of *Tretanorhinus* species

- Vidal N, Kindl SG, Wong A, Hedges SB. 2000. Phylogenetic relationships of xenodontine snakes inferred from 12S and 16S ribosomal RNA sequences. *Molecular Phylogenetics and Evolution* 14: 389–402.
- Villa J. 1969. Two new insular subspecies of the natricid snake *Tretanorhinus nigroluteus* Cope from Honduras and Nicaragua. *Journal of Herpetology* 3: 145–150.
- Villa J. 1970. Notas sobre la historia natural de la serpiente de los pantanos, *Tretanorhinus nigroluteus*. *Revista de Biología Tropical* 17: 97–104.
- Villa J. 1973. A snake in the diet of a kinosternid turtle. *Journal of Herpetology* 7: 380–381.
- Wilson LD, Hahn DE. 1973. The herpetofauna of the Islas de la Bahia, Honduras. *Bulletin of the Florida State Museum, Biological Sciences* 17: 93–150.



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Appendix 1. List of studies referring to *Tretanorhinus* species.

1. Alarcón-Pardo H. 1978. Primer registro de *Tretanorhinus nigroluteus nigroluteus* Cope (Reptilia: Serpentes: Colubridae) para Colombia. *Lozania* 27: 1–4.
2. Auth DL. 1994. Checklist and bibliography of the amphibians and reptiles of Panama. *Smithsonian Herpetological Information Service* 98: 1–59.
3. Barbour T. 1914. A contribution to the zoogeography of the West Indies, with special reference to amphibians and reptiles. *Memoirs of the Museum of Comparative Zoölogy at Harvard College* 44: 207–359.
4. Barbour T. 1916. The reptiles and amphibians of the Isle of Pines. *Annals of Carnegie Museum* 10: 207–308.
5. Barbour T; Amaral AD. 1924. Notes on some Central American snakes. *Occasional Papers of the Boston Society of Natural History* 5: 129–132.
6. Barbour T; Ramsden CT. 1916. Catalogo de los reptiles y anfibios de la Isla de Cuba. *Memorias de la Sociedad Cubana de Historia Natural "Felipe Poey" Habana* 2: 124–143.
7. Barbour T; Ramsden CT. 1919. The herpetology of Cuba. *Memoirs of the Museum of Comparative Zoölogy at Harvard College* 47: 69–213.
8. Barquero MD; Campos-Chinchilla P; Valverde R. 2005. *Tretanorhinus nigroluteus*: biology and captive maintenance of the Orangebelly Swamp Snake. *Reptilia* 42: 47–52.
9. Bocourt MF. 1891. Sur quelques ophidiens de l'Amerique intertropicale: appartenant au genre *Tretanorhinus*. *Le Naturaliste* 101: 121–122.
10. Boulenger GA. 1893. *Catalogue of the Snakes in the British Museum (Natural History)*. Volume I. Taylor and Francis, London, United Kingdom. 504 p.
11. Boulenger GA. 1903. Descriptions of new snakes in the collection of the British Museum. *Annals and Magazine of*

- Natural History: Zoology, Botany, and Geology* Series 7, 12: 350–354.
12. Cadle JE. 1984. Molecular systematics of Neotropical xenodontine snakes: II. Central American xenodontines. *Herpetologica* 40: 21–30.
 13. Cadle JE. 1985. The Neotropical colubrid snake fauna (Serpentes: Colubridae): Lineage components and biogeography. *Systematic Zoology* 34: 1–20.
 14. Campbell JA. 1999. *Amphibians and Reptiles of Northern Guatemala, the Yucatan, and Belize*. University of Oklahoma Press, Norman, Oklahoma, USA. 400 p.
 15. Castaño-M OV; Cárdenas-A G; Hernández-R EJ; Castro-H F. 2004. Reptiles en el Chocó biogeográfico. Pp. 599–632 In: *Diversidad Biótica IV. El Chocó Biogeográfico/Costa Pacífica*. Editor, Rangel-Ch JO. Universidad Nacional de Colombia, Instituto de Ciencias Naturales, Conservation International, Bogotá, Colombia. 997 p.
 16. Cisneros-Heredia DF. 2005. *Tretanorhinus mocquardi* (Swamp Snake). *Herpetological Review* 36: 340.
 17. Conant R. 1965. Miscellaneous notes and comments on toads, lizards, and snakes from Mexico. *American Museum Novitates* 2205: 1–38.
 18. Cope ED. 1861. Contributions to the ophiology of Lower California, Mexico, and Central America. *Proceedings of the Academy of Natural Sciences of Philadelphia* 13: 292–306.
 19. Crother BI. 1989. A redescription of the hemipenis of *Hydromorphus concolor* (Colubridae) with comments on its tribal allocation. *Copeia* 1989: 227–229.
 20. Crother BI. 1999. Phylogenetic relationships among West Indian xenodontine snakes (Serpentes; Colubridae) with comments on the phylogeny of some mainland xenodontines. *Contemporary Herpetology* 2: 7–30.
 21. Daniel H. 1949. Las serpientes de Colombia. *Revista Facultad Nacional de Agronomía Medellín* 10: 301–333.
 22. Dowling HG; Hass CA; Hedges SB; Highton R. 1996. Snake relationships revealed by slow-evolving proteins: a preliminary survey. *Journal of Zoology* 240: 1–28.
 23. Duellman WE. 1963. Amphibians and reptiles of the rainforests of southern El Petén, Guatemala. *University of Kansas Publications, Museum of Natural History* 15: 205–249.
 24. Duméril AMC; Bibron G; Duméril A. 1854. *Erpétologie Générale ou Histoire Naturelle Complète des Reptiles*. Volume 7 (partie 1). Librairie Encyclopédique de Roret, Paris, France. 780 p.
 25. Dunn ER. 1932. The colubrid snakes of the Greater Antilles. *Copeia* 1932: 89–92.
 26. Dunn ER. 1939. Mainland forms of the snake genus *Tretanorhinus*. *Copeia* 1939: 212–217.
 27. Estrella-Morales J; Piedra-Castro L. 2018. Anfíbios y reptiles (herpetofauna) en las asociaciones vegetales de la Laguna de Gandoca, Limón, Costa Rica. *Tecnología en Marcha* 31: 127–135.
 28. García-Balderas CM; Carbajal-Márquez RA; Cedeño-Vázquez JR. 2016. Distribution notes. *Tretanorhinus nigroluteus* Cope, 1861. *Mesoamerican Herpetology* 3: 200.
 29. Garman S. 1883. The reptiles and batrachians of North America. *Memoirs of the Museum of Comparative Zoölogy at Harvard College* 3: 1–219.
 30. González-C A; Rodríguez-S L; Rivalta-G V. 2014. Colección herpetológica del Instituto de Ecología y Sistemática, La Habana, Cuba: Familia Dipsadidae, géneros *Caraiba* y *Tretanorhinus*; familia Natricidae, género *Nerodia*. *Revista Colombiana de Ciencia Animal* 6: 38–54.
 31. González-Sánchez VH; Johnson JD; García-Padilla E; Mata-Silva V; DeSantis DL; Wilson LD. 2017. The herpetofauna of the Mexican Yucatan Peninsula: composition, distribution, and conservation status. *Mesoamerican Herpetology* 4: 263–380.
 32. Grant C. 1941. The herpetology of the Cayman Islands. *Bulletin of the Institute of Jamaica, Science Series* 2: 1–56.
 33. Grant C. 1949. Notes on *Tretanorhinus* in Cuba and the Isle of Pines. *Journal of Agriculture of the University of Puerto Rico* 30: 102–117.
 34. Grazziotin FG; Zaher H; Murphy RW; Scrocchi G; Benavides MA; Zhang YP; Bonatto SL. 2012. Molecular phylogeny of the New World Dipsadidae (Serpentes: Colubroidea): a reappraisal. *Cladistics* 28: 437–459.
 35. Gundlach J. 1880. *Contribucion a la Erpetologia Cubana*. G. Montiel y Ca, Habana, Cuba. 99 p.
 36. Günther ACLG. 1885–1902. *Biologia Centrali-Americana. Reptilia and Batrachia*. F. Du Cane Godman and Osbert Savin, London, United Kingdom. 7 Volumes.
 37. Hedges SB. 1996. The origin of West Indian amphibians and reptiles. Pp. 95–128 In: *Contributions to West Indian Herpetology: a Tribute to Albert Schwartz*. Editors, Powell R, Henderson RW. Society for the Study of Amphibians and Reptiles, Ithaca, New York, USA. 457 p.
 38. Henderson RW; Hoevers LG. 1977. The seasonal incidence of snakes at a locality in northern Belize. *Copeia* 1977: 349–355.
 39. Henderson RW; Hoevers LG. 1979. Variation in the snake *Tretanorhinus nigroluteus lateralis* in Belize with notes on breeding tubercles. *Herpetologica* 35: 245–248.
 40. Hernández-Ordóñez O; Arroyo-Rodríguez V; González-Hernández A; Russildi G; Luna-Reyes R; Martínez-Ramos M; Reynoso VH. 2015. Range extensions of amphibians and reptiles in the southeastern part of the Lacandona rainforest, Mexico. *Revista Mexicana de Biodiversidad* 86: 457–468.
 41. Highton R; Hedges SB; Hass CA; Dowling HG. 2002. Snake relationships revealed by slowly-evolving proteins: further analysis and a reply. *Herpetologica* 58: 270–275.
 42. Jan G. 1865. Enumerazione sistematica degli ofidi appartenenti ai gruppo Potamophilidae. *Archive per la Zoologia, l'Anatomia et la Fisiologia* 3: 201–265.

43. Johnson JD; Mata-Silva V; García Padilla E; Wilson LD. 2015. The herpetofauna of Chiapas, Mexico: composition, distribution, and conservation. *Mesoamerican Herpetology* 2: 271–329.
44. Köhler G. 2008. *Reptiles of Central America*. 2nd edition. Herpeton-Verlag, Offenbach, Germany. 400 p.
45. Köhler G; Cedeño-Vázquez JR; Beutelspacher-García PM. 2016. The Chetumal Snake Census: generating biological data from road-killed snakes. Part 1. Introduction and identification key to the snakes of southern Quintana Roo, Mexico. *Mesoamerican Herpetology* 3: 669–687.
46. Lee JC. 2000. *A Field Guide to the Amphibians and Reptiles of the Maya World*. Cornell University Press, Ithaca, New York, USA. 416 p.
47. Malnate EV. 1972. Observations on the vertebral hypapophyses and associated musculature in some snakes, with special reference to the Colubridae. *Zoologische Mededelingen* 47: 225–239.
48. Marx H; Rabb GB. 1972. Phyletic analysis of fifty characters of advanced snakes. *Fieldiana Zoology* 63: 1–321.
49. Mata-Silva V; Johnson JD; Wilson LD; García-Padilla E. 2015. The herpetofauna of Oaxaca, Mexico: composition, physiographic distribution, and conservation status. *Mesoamerican Herpetology* 2: 5–62.
50. McCoy CJ. 1970. The snake fauna of Middlesex, British Honduras. *Journal of Herpetology* 4: 135–140.
51. McCranie JR. 2011. *The Snakes of Honduras. Systematics, Distribution, and Conservation*. Society for the Study of Amphibians and Reptiles, Ithaca, New York, USA. 714 p.
52. Mead JI; Steadman DW. 2017. Late Pleistocene snakes (Squamata: Serpentes) from Abaco, The Bahamas. *Geobios* 50: 431–440.
53. Meyer JR. 1966. Records and observations on some amphibians and reptiles from Honduras. *Herpetologica* 22: 172–181.
54. Minton SA Jr. 1976. Serological relationships among some congeneric North American and Eurasian colubrid snakes. *Copeia* 1976: 672–678.
55. Neill WT. 1958. The occurrence of amphibians and reptiles in saltwater areas, and a bibliography. *Bulletin of Marine Science of the Gulf and Caribbean* 8: 1–97.
56. Neill WT. 1965. Notes on aquatic snakes, *Natrix* and *Tretanorhinus*, in Cuba. *Herpetologica* 21: 172–181.
57. Peters JA. 1960. The snakes of Ecuador; check list and key. *Bulletin of the Museum of Comparative Zoölogy at Harvard College* 122: 489–541.
58. Peters JA; Orejas-Miranda B. 1970. Catalogue of the Neotropical Squamata. Part I. Snakes. *Bulletin United States National Museum* 297: 1–347.
59. Peters JL; Allen GM; Barbour T; Loveridge A. 1929. Vertebrates from the Corn Islands. *Bulletin of the Museum of Comparative Zoölogy at Harvard College* 69: 125–146.
60. Petzold HG. 1967. Some remarks on the breeding biology and the keeping of *Tretanorhinus variabilis*, a water snake of Cuba. *Herpetologica* 23: 242–246.
61. Pinou T; Dowling HG. 1994. The phylogenetic relationships of the Central American snake *Tretanorhinus*: data from morphology and karyology. *Amphibia-Reptilia* 15: 297–305.
62. Ray JM; Ruback P. 2015. Updated checklists of snakes for the provinces of Panamá and Panamá Oeste, Republic of Panama. *Mesoamerican Herpetology* 2: 167–188.
63. Rodríguez Schettino L; Mancina CA; Rivalta González V. 2013. Reptiles of Cuba: checklist and geographic distributions. *Smithsonian Herpetological Information Service* 144: 1–96.
64. Rosén N. 1905. List of the snakes in the zoological museums of Lund and Malmö, with descriptions of new species and a new genus. *Annals and Magazine of Natural History; Zoology, Botany, and Geology* Series 7, 15: 168–181.
65. Sánchez-C H; Castaño-M O; Cárdenas-A G. 1995. Diversidad de los reptiles en Colombia. Pp. 277–326 In: *Colombia Diversidad Biótica I*. Editor, Rangel-Ch JO. Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Inderena, Bogotá, Colombia. 442 p.
66. Savage JM. 2002. *The Amphibians and Reptiles of Costa Rica: a Herpetofauna between Two Continents, between Two Seas*. University of Chicago Press, Chicago, Illinois, USA. 954 p.
67. Schmidt KP. 1935. Amphibians and reptiles collected by the Smithsonian Biological Survey of the Panama Canal Zone. *Smithsonian Miscellaneous Collections* 89: 1–20.
68. Schmidt KP. 1941. The amphibians and reptiles of British Honduras. *Field Museum of Natural History, Zoological Series* 22: 475–510.
69. Schwartz A; Henderson RW. 1991. *Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History*. University of Florida Press, Gainesville, Florida, USA. 720 p.
70. Schwartz A; Ogren LH. 1956. A collection of reptiles and amphibians from Cuba, with the descriptions of two new forms. *Herpetologica* 12: 91–110.
71. Seidel ME; Franz R. 1994. Amphibians and reptiles (exclusive of marine turtles) of the Cayman Islands. Pp. 407–433 In: *The Cayman Islands: Natural History and Biogeography*. Editors, Brunt MA, Davies JE. Kluwer Academic Publishers, Dordrecht, Netherlands. 604 p.
72. Smith HM. 1965. Two new colubrid snakes from the United States and Mexico. *Journal of the Ohio Herpetological Society* 5: 1–4.
73. Smith HM; Taylor EH. 1945. An annotated checklist and key to the snakes of Mexico. *Bulletin United States National Museum* 187: 1–239.
74. Solís JM; Wilson LD; Townsend JH. 2014. An updated list of the amphibians and reptiles of Honduras, with

- comments on their nomenclature. *Mesoamerican Herpetology* 1: 122–144.
75. Solórzano A. 2004. *Serpientes de Costa Rica: Distribución, Taxonomía, e Historia Natural = Snakes of Costa Rica: Distribution, Taxonomy, and Natural History*. Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, Costa Rica. 791 p.
76. Stuart LC. 1937. Some further notes on the amphibians and reptiles of the Peten forest of northern Guatemala. *Copeia* 1937: 67–70.
77. Sunyer J. 2014. An updated checklist of the amphibians and reptiles of Nicaragua. *Mesoamerican Herpetology* 1: 185–202.
78. Taylor EH. 1951. A brief review of the snakes of Costa Rica. *The University of Kansas Science Bulletin* 34: 3–188.
79. Torres-Carvajal O; Salazar-Valenzuela D; Merino-Viteri A. 2014. ReptiliaWebEcuador, Versión 2014.0. Museo de Zoología QCAZ, Pontificia Universidad Católica del Ecuador, Quito, Ecuador. Available: <http://zoologia.puce.edu.ec/Vertebrados/reptiles/reptilesEcuador> [Accessed: 4 March 2015].
80. Vaeth RH; Rossman DA; Shoop W. 1985. Observations of tooth surface morphology in snakes. *Journal of Herpetology* 19: 20–26.
81. Vidal N; Kindl SG; Wong A; Hedges SB. 2000. Phylogenetic relationships of xenodontine snakes inferred from 12S and 16S ribosomal RNA sequences. *Molecular Phylogenetics and Evolution* 14: 389–402.
82. Villa J. 1969. Two new insular subspecies of the natricid snake *Tretanorhinus nigroluteus* Cope from Honduras and Nicaragua. *Journal of Herpetology* 3: 145–150.
83. Villa J. 1970. Notas sobre la historia natural de la serpiente de los pantanos, *Tretanorhinus nigroluteus*. *Revista de Biología Tropical* 17: 97–104.
84. Villa J. 1973. A snake in the diet of a kinosternid turtle. *Journal of Herpetology* 7: 380–381.
85. Wilson LD; Hahn DE. 1973. The herpetofauna of the Islas de la Bahia, Honduras. *Bulletin of the Florida State Museum, Biological Sciences* 17: 93–150.
86. Wood GC. 1939. The genus *Tretanorhinus* in Cuba and the Isle of Pines. *Proceedings of the New England Zoölogical Club* 18: 5–11.
87. Zaher H; de Oliveira L; Graziotin FG; Campagner M; Jared C; Antoniazzi MM; Prudente AL. 2014. Consuming viscous prey: a novel protein-secreting delivery system in neotropical snail-eating snakes. *BMC Evolutionary Biology* 14: 58–86.

Appendix 2. Number of specimens of *Tretanorhinus* held in each scientific collection from which data were obtained.

Specimens	Abbrev	Institution name
7	ANSP	Academy of Natural Sciences, Philadelphia
143	AMNH	American Museum of Natural History
1	BYU	Bingham Young University, Monte L. Bean Life Science Museum
59	CAS	California Academy of Sciences
12	CM	Carnegie Museum of Natural History
2	CHP	Círculo Herpetológico de Panamá
12	FMNH	Field Museum of Natural History
82	FLMNH	Florida Museum of Natural History
6	FHGO	Fundación Herpetológica Gustavo Orcés
12	INHS	Illinois Natural History Survey, University of Illinois (formerly University of Illinois Museum of Natural History [UIMNH])
5	IBUNAM	Instituto de Biología, Universidad Nacional Autónoma de México
8	IAvH	Instituto de Investigación de Recursos Biológicos Alexander von Humboldt
35	LACM	Los Angeles County Museum of Natural History
64	LSUMZ	Louisiana Museum of Natural History (formerly Louisiana State University, Museum of Zoology)
45	MPM	Milwaukee Public Museum
15	MNHN	Muséum National d'Histoire Naturelle
60	MCZ	Museum of Comparative Zoology, Harvard University
3	MVZ	Museum of Vertebrate Zoology, University of California at Berkeley
52	USNM	National Museum of Natural History, Smithsonian Institution (formerly United States National Museum)
11	BMNH	Natural History Museum (formerly British Museum of Natural History)
5	RBINS	Royal Belgian Institute of Natural Sciences
3	ROM	Royal Ontario Museum
3	TCWC	Texas Cooperative Wildlife Collection
2	MHUA	Universidad de Antioquia, Museo de Herpetología
12	MZUCR	Universidad de Costa Rica, Museo de Zoología
2	UVC	Universidad del Valle
1	UTCH	Universidad Tecnológica del Chocó
2	UCM	University of Colorado, Museum of Natural History
77	KU	University of Kansas, Biodiversity Institute
10	UMMZ	University of Michigan, Museum of Zoology
4	UTA	University of Texas at Arlington
755	Total	



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Three new species of *Hemidactylus* Oken, 1817 (Squamata, Gekkonidae) from Iran

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Abstract.—Based on morphological characters, three new species of the genus *Hemidactylus* are described, one from the Zagros Mountains (Khuzestan Province) and two from the coastal Persian Gulf (Bushehr Province) of Iran. The three new species can be differentiated from all other *Hemidactylus* inhabitants of Iran and adjacent area congeners by distinct morphometric, meristic, and color characters. Comparisons with other species of *Hemidactylus* are presented and a key to the genus is provided. Some information about the ecology, biology, and conservation of the three new species is provided. Existing data suggest these geckos are point endemics. Some additional historical information about the *Hemidactylus* inhabitants of Iran is discussed, particularly *H. parkeri*.

Keywords. Bushehr, *Hemidactylus achaemenidicus* sp.n., *H. pseudoromeshkanicus* sp.n., *H. sassanidianus* sp.n., *H. parkeri*, Khuzestan, Sauria, Reptilia

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Introduction

Globally, the gekkonid genus *Hemidactylus* Oken, 1817 currently consists of 154 species distributed across all tropical and subtropical continental landmasses, including intervening oceanic and continental islands (Carranza and Arnold 2012; Šmíd et al. 2013a,b, 2015; Uetz 2019). Four families and 70 species of geckos occur in Iran: 50 species of Gekkonidae, 10 species Phyllodactylidae, seven species of Sphaerodactylidae, and three species of Eublepharidae (Uetz 2019). The four *Hemidactylus* species reported so far from Iran are: *H. flaviviridis*, *H. persicus*, *H. robustus*, and *H. romeshkanicus* (Anderson 1999; Bauer et al. 2006a; Rastegar-Pouyani et al. 2006; Torki et al. 2011; Kamali 2013; Šmíd et al. 2014). Only one of them is endemic to Iran (*H. romeshkanicus*). During a 2007–2010 collection program in south-western Iran, from the Zagros Mountains and coastal Persian Gulf, several geckos were collected which, upon laboratory examination, were found to differ in important characters from Iranian geckos already known.

In this article, they are described morphologically and compared to the previously known *Hemidactylus* species from Iran, as well as those from neighboring

regions. Additionally, two notes regarding *H. parkeri* are presented, and comments on the conservation of geckos in Iran are provided.

Materials and Methods

During several field trips in the Iranian plateau, three new *Hemidactylus* species were collected from this region (Fig. 1): (a) Kangan region, near the coastal Persian Gulf, Bushehr province, (b) Tangestan region, Bushehr province, and (c) Kole-Saat, Khuzestan province. All specimens of the three new species were assigned catalog numbers for the ZFMK (Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany); and FTHM (Farhang Torki Herpetological Museum, Nourabad City, Iran), with the latter deposited in Farhang Torki Ecology and Herpetology Center for Research (FTEHCR).

The taxonomic characters of *Hemidactylus* species from Iran are not well defined. For most species, no museum specimens were available for comparison. Rather, published descriptions of geckos known from Iran were compared to the morphological characters of the newly collected material (e.g., Moravec et al. 2011;

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Fig. 1. Type localities of three new geckos in Iran.

Carranza and Arnold 2012; Šmíd et al. 2013a, 2015). For comparison with *H. romeshkanicus* ZMB 75020 from the Museum für Naturkunde, Leibniz Institut für Biodiversitäts- und Evolutionsforschung zu Berlin (formerly Zoologisches Museum Berlin, Germany) was used. For comparison with *Hemidactylus* spp. distributed outside Iran, original descriptions or other publications containing morphological analyses of *Hemidactylus* species were used (e.g., Anderson 1999; Giri et al. 2003; Baha el Din 2003, 2005; Bauer et al. 2006a,b, 2007; Sindaco et al. 2007; Giri and Bauer 2008; Giri 2008; Mahony 2010; Agarwal et al. 2011; Busais and Joger 2011; Moravec et al. 2011; Torki et al. 2011; Mirza and Rajesh 2014; Vasconcelos and Carranza 2014; Carranza and Arnold 2012; Šmíd et al. 2013a, 2015; Safaei-Mahroo et al. 2017).

Characters were selected to optimize comparisons with data reported by Moravec et al. (2011), Carranza and Arnold (2012), Wagner et al. (2014), Vasconcelos and Carranza (2014), and Šmíd et al. (2013a, 2015). Measurements were taken using a dial caliper with 0.01 mm precision. Additionally, other characters important for the taxonomy of *Hemidactylus* were used, such as nasals in contact and 1st postmental in contact with 2nd lower labial (e.g., Moravec et al. 2011; Šmíd et al. 2013). Characters used to describe the three new *Hemidactylus* are as follows: SVL: snout-vent length; TRL: trunk length; TL: tail length; R1: TL/SVL; HL: head length; HW: head width; HH: head height; R2: HL/

SVL; R3: HW/HL; R4: HH/HL; OD: orbital diameter; NE: nares to eye distance; IN: internarial distance; IO1: anterior interorbital distance; IO2: posterior interorbital distance; TB: longitudinal tubercle rows; PAP: number of precloacal pores; SL (L/R): number of supralabials; IL (L/R): number of infralabial scales; LP1 (L/R): number of lamellae under the first finger of the pes; LP4 (L/R): number of lamellae under the fourth finger of the pes; FP: femoral pores; and PM: postmentals. Abbreviations used in tables are as follows: M: male; F: female; T: total; A: ANOVA test; F: one-way ANOVA *F* value; dF: degrees of freedom; P: probability; DM: Difference of means; and DD: Direction of difference.

Because of the absence of sexual size dimorphism in the arid clade of *Hemidactylus* (Carranza and Arnold 2012), both sexes were analyzed together. Statistical procedures used to test for differences between the sexes included one-way ANOVA (at 95% confidence level [$P < 0.05$]) and Principal Component Analysis (PCA).

Taxonomy

Hemidactylus achaemenidicus sp.n. (Figs. 2–5)

Hemidactylus turcicus - Torki et al. 2011

Hemidactylus persicus - Carranza and Arnold 2012

Hemidactylus persicus - Šmíd et al. 2013

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Fig. 2. Dorsal and ventral views of (a, b) holotype and (c, d) paratype specimens of *Hemidactylus achaemenidicus* sp.n.

Holotype

ZFMK 98567, adult male, collected at the end of the southern Zagros Mountains, Kangan, Bushehr Province, Southern Iran, on 10 May 2008 (27°18'N, 52°42'E, 50–221 m asl).

Paratypes

ZFMK 97750–97753; ZFMK 98568–73; and FTHM 005110, six adult male specimens (ZFMK 97750–97752; ZFMK 98569–70; FTHM 005110), and four adult female specimens (ZFMK 97753; ZFMK 98568, 71, 72), same data as for holotype.

Diagnosis

A small sized *Hemidactylus*, maximum snout-vent length 39.8 mm; tubercles distributed over the entire dorsum (except for forelimbs); granules cover head and extend to neck; tubercle rugosity dimorphism occurs between males and females over dorsal body, limbs, and tail (males have more rugose tubercles than females); proximal portion of tail (ventral view) covered by small scales without femoral pores; precloacal pores present; six tubercles on most whorls of tail; two postmentals; low number of lamellae under pes; subcaudal scales started more distally (approximately after proximal one-third of tail), only a few subcaudals (plate-like) in original tail (0–22), that started so far as anal; proximal dorsal tail covered by regular whorls of tubercles (keeled in male and plate-like in female); ventral scales not imbricate; the ends of ventral scales are denticulated; enlarged scansors beneath fingers, scansors are mostly divided, terminal scansor is single; dorsal color pattern shows much variability (regular or irregular crossbars, longitudinal bands, large or small spots), and this is true for the tail (regular or irregular bars, large and small spots), venters of all specimens are without spots (uniform).

Description of Holotype (Figs. 2–3)

Measurements (in mm): body size: 39.8; tail length: 40.5; interlimbs: 18.3; head width: 7.3; head length: 11.7; head depth: 4.9; eye-eye: 4.7; ear opening: 0.82; eye diameter: 3.0; forelimb length: 12.3; hindlimb length: 15.5.



Fig. 3. (a) Postmentals and (b) precloacal pores in holotype of *Hemidactylus achaemenidicus* sp.n.

Body depressed, tail more or less flattened; head triangular-shaped; two postmentals, 1st postmentals enlarged and in contact, 2nd postmentals behind the first enlarged postmentals, the 1st postmentals in contact with the 1st infralabials, the 2nd left postmental distinct from infralabials by one series of scales, the 2nd right postmentals in contact with the 2nd infralabials (and weakly with the 1st), four scales between 2nd postmentals; Infralabials: eight; supralabials: nine; nostril surrounded by five scales (the 1st supralabial, rostral, three small on posterior); nasals not in contact and separated by one scale; ear openings more or less falcate-shaped, and horizontal; 14 scales between nostril and eye; 24 scales between eye and ear; rostrum covered by large granules; space between eyes covered by 27 small granules, and 10 small simple tubercles distributed among them; upper head covered by smallest granules and many small simple tubercles distributed among them; tubercles on upper ears and behind eyes are simple; tubercles on occiput mostly simple and less pointed; tubercles on neck are pointed and keeled (heterogeneous); from rostrum to neck body covered by granules; tubercles distributed on dorsum, head, and limbs; tubercles not found on arm; most body tubercles are keeled; dorsal tubercles are strongly keeled,

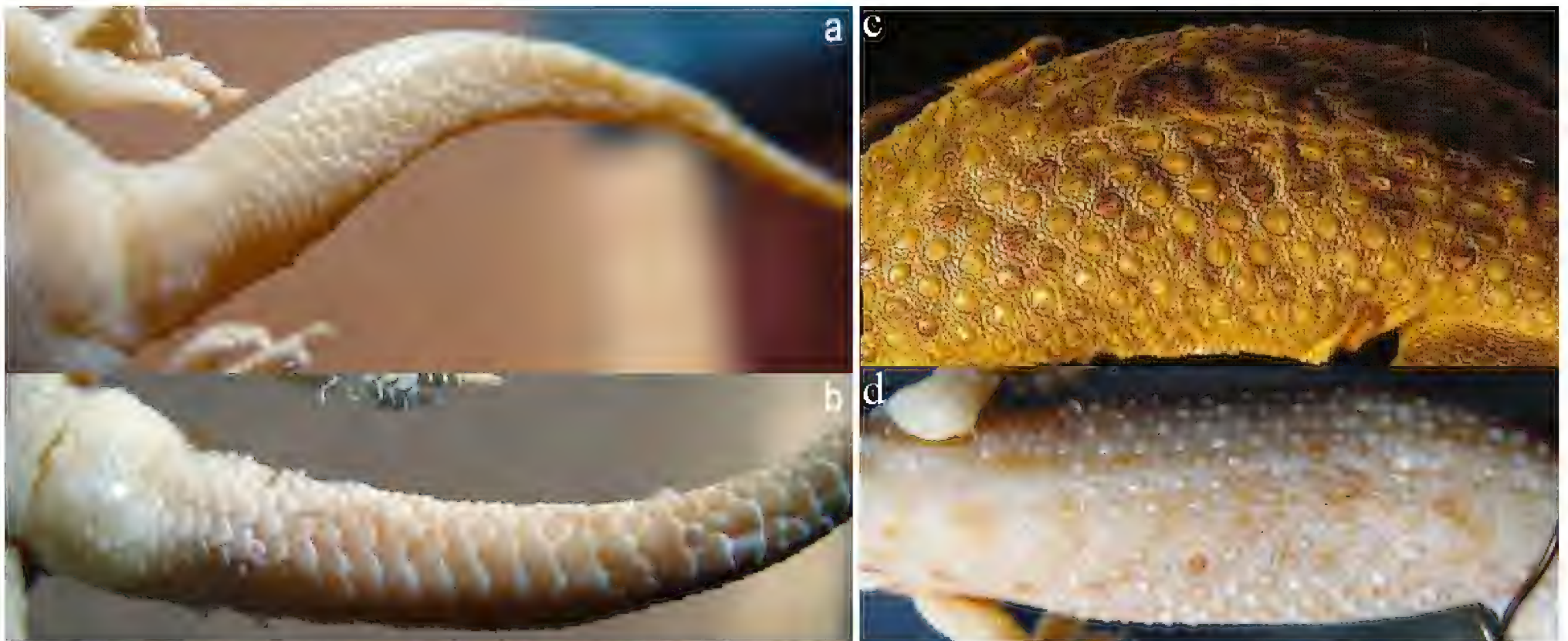


Fig. 4. Subcaudal of tail of (a) ZFMK97753 and (b) ZFMK 98567 of *Hemidactylus achaemenidicus* sp.n. Comparison of dorsal body between (c) holotype of *H. achaemenidicus* sp.n. and (d) lectotype of *H. robustus*. Photo from Šmíd et al. 2015.

tubercles on proximal of back surrounded by 11–12 scales (middle: 12–13, distal: 13), dorsal tubercles do not show regular form and abnormalities occur in a few points (intermixed with some small simple tubercles); enlarged, trihedral, and strongly keeled tubercles distributed on distal part of dorsum (between hindlimbs) as well as nearest to tail; tubercles on forearm are simple; tubercles on femur heterogeneous (simple, pointed, and keeled); foreleg tubercles heterogeneous in size and shape (pointed and keeled); size of the tubercles on limbs is different and is as follows: foreleg > femur > forearm; scales on palm and sole are granule-like; 17 rows (mostly regular) of tubercles on back; 21 tubercles between interlimbs.

Tail is original; first part of tail (one-third) covered by small scales, subcaudal plates cover following third, less than 12 scales (moderate size: 50% of tail width, not imbricate) on subcaudal, last part; distal one-third of tail is without subcaudals (covered by small scales); without crossbars on dorsum of tail, small irregular spots present in first half of tail; tubercle whorls only found on first half of tail, 1st to 6th whorls more or less irregular and separated by one scale, includes six large, trihedral, and strongly keeled tubercles, after them real whorls start: six tubercles in 1st to 3rd whorl, four for 4th to 7th, first whorl separated from secondary by two scales, four scales between 2nd–3rd and 3rd–4th, six scales between 4th–5th, five between 5th–6th and 6th–7th, after them tubercles converted to scales; seven (3+1+3) precloacal pores; no femoral pores; enlarged scansors are plate-like; terminal scansor is single; lamellae on fingers as follows: 1st: five, 2nd: seven, 3rd: seven, 4th: seven, 5th: eight; lamellae on pes as follows: 1st: six, 2nd: eight, 3rd: nine, 4th: 10, 5th: eight; claws in front of scansors. Palm and sole covered by granule-like scales.

Coloration of upper head is covered by longitudinal discontinuous rows that extended to neck, and are

irregular onto dorsal body; dorsum without bars; few irregular bars and small spots cover dorsum of tail; one bar between nostril-eye-ear; venter of body, limbs, and tail uniformly without pattern; pattern in preserved specimen is similar to the live specimen and all spots and bars are obvious; the preserved specimen is colorless.

Variation (Fig. 4a–b)

Some variation among paratypes is described as follows: tubercles distributed all over dorsum (except arms); granules cover head and extend to neck. Tubercle rugosity differed between males and females on overall dorsal body, limbs, and tail (males with more strongly rugose tubercles than females), females have wide (approximately flattened shape) dorsal tubercles and males have extended trihedral tubercles. Proximal tail in most specimens is cycloid and ventral view covered by small scales (same as dorsum); proximal tail (dorsal view) covered by 4–6 irregular whorls of tubercles (strongly keeled) separated by one scale, followed by regular whorls of six tubercles in each whorl, started and separated by 2–6 scales, more than six regular whorls are obvious in all specimens (first half) and do not continue to posterior half of tail (tubercles converted to scales). Number of precloacal pores is variable as follows: six (five specimens), seven (holotype), and eight (ZFMK97751). Most specimens have two postmentals, postmentals in all specimens are not uniform and variability is as follows: one specimen (ZFMK 98570) has five postmentals (left+right), two anterior, two posterior, and one large scale between anteriors; anteriors not in contact with one another and in contact with 1st and 2nd infralabials; seven specimens have normal postmentals and anterior in all specimens in contact with 1st and 2nd infralabials; in one specimen (FTHM005110) 2nd postmentals (left and right) separated from infralabials by one series of scales, and 1st postmentals in contact with 1st infralabial; left 2nd



Fig. 5. Type locality of *Hemidactylus achaemenidicus* sp.n., Kangan, Bushehr, southern Iran.

postmental in ZFMK97751 separated from infralabials by one series of scales, and 1st postmentals are in contact with 1st infralabials; finally left 1st postmental of ZFMK 98571 contacts 1st infralabials. Subcaudal scales begin approximately after first third of tail, a lesser number of subcaudals (plate like) in original tail (0 to 22); first half of dorsal tail covered by regular whorls of tubercles (strongly keeled in males and plate-like in females). Dorsal color pattern is variable (regular or irregular cross bars, longitudinal band, large or small spots), this is true for tail (regular or irregular bars, large and small spots), venter of all specimens is uniform, without spots; venter in live specimens is white and tail is yellowish or dark, in preserved specimens ventral is yellowish and ventral of tail is darkish. More data on the variation are shown in Table 1.

Sexual dimorphism is evident. In general, males show larger body size and head size than females (Table 1).

Based on statistical analysis three characters, TRL, IO2, and LP4R, are significantly different between the sexes as follows: males have significantly ($P = 0.03$, $f = 6.21$) larger trunk length than females (16.9 ± 0.37 vs. 14.3 ± 1.28); this is true for IO2 and in males (4.70 ± 0.1) is significantly ($P = 0.03$, $f = 6.09$) larger than in females (4.22 ± 0.18). In contrast, number of lamellae under 4th pes (right side) in females (10 ± 0.0) is significantly ($P = 0.01$, $f = 8.18$) greater than in males (9.28 ± 0.18). Five characters (SVL, TRL, TL, HW, HL) in females show much more variability than in males; in contrast, three characters (OD, NE, IN) in males are much more variable than in females. All females have 16 dorsal tubercle rows, and in males they number 16 or 17 (16.4 ± 0.2). Lamellar variability under 1st and 4th finger of pes in females is zero and in males is one (except ILL: female is one and males are zero). More data on the dimorphism are shown in Table 1.

Habitat and Ecology (Fig. 5)

Hemidactylus achaemenidicus sp.n. are distributed in the eastern part of Bushehr Province (edge of Hormozgan Province), in Kangan, Assaloye City. The habitat of *H. achaemenidicus* sp.n. is flat land covered by Jujube trees (*Ziziphus jujuba*). The type locality is located in the northern part of the Persian Gulf. A few lizard and snake species were observed at the type locality: *Trapelus agilis*, *Laudakia nupta*, and *Echis carinatus*.

Distribution

So far, the species is only known from the type locality.

Etymology

The species name “*achaemenidicus*” refers to “The Achaemenid Empire,” also called the First Persian Empire. It was an empire based in Western Asia, founded by Cyrus the Great, and notable for including various civilizations and becoming the largest empire at that time.

Comparisons

Based on a phylogenetic study of one paratype specimen (FTHM 005100 is erroneous and FTHM 005110 is the true code; also the locality cited in the phylogeny section must be changed to the type locality of the new species) *H. achaemenidicus* sp.n. is completely distinct from *H. robustus*, *H. turcicus*, and other recently described species inhabiting Oman (see phylogram of Carranza and Arnold 2012; Šmíd et al. 2015). *Hemidactylus achaemenidicus* sp.n. was compared with the re-description of *H. robustus* Šmíd et al. (2015) [see Table 2]. *Hemidactylus achaemenidicus* sp.n. is different from *H. robustus* by smaller body size in males (36.5 ± 0.9 mm vs. 41.8 ± 2.3) and females (33.1 ± 2.0 mm vs. 43.6 ± 4.7), more longitudinal tubercle rows (16.2 ± 0.1 vs. 14.8 ± 1.2), and keeled (vs. weakly keeled and posteriorly pointed) as well as rugosity dimorphism (quite distinct

Table 1. Measurements and scale counts of *Hemidactylus achaemenidicus* **sp.n.** Abbreviations: SVL: snout-vent length; TRL: trunk length; TL: tail length; R1: TL/SVL; HL: head length; HW: head width; HH: head height; R2: HL/SVL; R3: HW/HL; R4: HH/HL; OD: orbital diameter; NE: nares to eye distance; IN: internarial distance; IO1: anterior interorbital distance; IO2: posterior interorbital distance; TB: longitudinal tubercle rows; PAP: number of preanal pores; SL (L/R): number of supralabials; IL (L/R): number of infralabial scales; LP1 (L/R): number of lamellae under the first finger of the pes; LP4 (L/R): number of lamellae under the fourth finger of the pes; M: male; F: female; T: total; A: ANOVA test; F: one-way ANOVA *F* value; dF: degree of freedom; P: probability (following Carranza and Arnold 2012); DM: difference of means; DD: direction of difference.

		SVL	TRL	TL	R1	HL	HW	HH	R2	R3	R4	OD	NE	IN	IO1	IO2	TB	PAP	SLL	SLR	ILL	ILR	LP1L	LP1R	LP4L	LP4R	
M	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4		
	Mean	36.5	16.9	38.5	1.03	10.9	6.91	4.68	0.29	0.62	0.42	2.55	2.91	0.97	3.22	4.70	16.4	6.42	9.42	9.71	8.00	7.85	5.57	5.71	9.42	9.28	
	SEM	0.92	0.37	1.48	0.01	0.28	0.17	0.11	0.00	0.01	0.01	0.11	0.11	0.07	0.08	0.10	0.20	0.29	0.20	0.18	0.00	0.14	0.20	0.18	0.20	0.18	
	Max	39.8	18.3	41.4	1.09	12.2	7.40	5.00	0.31	0.68	0.47	3.00	3.40	1.30	3.40	5.00	17	8.00	10.0	10.0	8.00	8.00	6.00	6.00	10.0	10.0	
	Min	32.9	15.8	34.9	1.01	10.2	6.20	4.30	0.27	0.60	0.38	2.10	2.50	0.80	2.80	4.30	16	6.00	9.00	9.00	8.00	7.00	5.00	5.00	9.00	9.00	
R	6.90	2.50	6.50	0.08	2.00	1.20	0.70	0.04	0.08	0.09	0.09	0.90	0.90	0.50	0.60	0.70	1.00	2.00	1.00	1.00	0.00	1.00	1.00	1.00	1.0	1.00	
F	N	7	7	4	4	7	7	7	7	7	7	7	7	7	7	7	7		7	7	7	7	7	7	7	7	
	Mean	33.1	14.3	31.3	0.93	9.95	6.30	4.25	0.29	0.62	0.42	2.30	2.70	0.97	2.90	4.22	16.0		9.75	9.25	7.75	7.75	6.00	6.00	10.0	10.0	
	SEM	2.05	1.28	3.10	0.05	0.51	0.44	0.35	0.00	0.01	0.01	0.07	0.12	0.04	0.14	0.18	0.00		0.25	0.25	0.25	0.25	0.00	0.00	0.00	0.00	
	Max	38.5	17.4	38.8	1.07	11.1	7.50	5.30	0.30	0.67	0.47	2.50	3.00	1.10	3.20	4.70	16.0		10.0	10.0	8.00	8.00	6.00	6.00	10.0	10.0	
	Min	28.9	11.5	24.5	0.84	8.70	5.40	3.70	0.29	0.60	0.39	2.20	2.40	0.90	2.60	3.80	16.0		9.00	9.00	7.00	7.00	6.00	6.00	10.0	10.0	
R	9.60	5.90	14.3	0.23	2.40	2.10	1.60	0.01	0.07	0.08	0.30	0.60	0.60	0.20	0.60	0.90	0.00		1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	
T	N	11	11	8	8	11	11	11	11	11	11	11	11	11	11	11	11	4	11	11	11	11	11	11	11	11	
	Mean	35.3	15.9	34.9	0.98	10.6	6.69	4.52	0.29	0.62	0.42	2.46	2.83	0.97	3.10	4.52	16.27	6.42	9.54	9.54	7.90	7.81	5.72	5.81	9.63	9.54	
	SEM	1.03	0.62	2.10	0.03	0.28	0.20	0.15	0.00	0.00	0.00	0.08	0.08	0.04	0.08	0.11	0.14	0.29	0.15	0.15	0.09	0.12	0.14	0.12	0.15	0.15	
	Max	39.8	18.3	41.4	1.09	12.2	7.50	5.30	0.31	0.68	0.47	3.00	3.40	1.30	3.40	5.00	17.0	8.00	10.0	10.0	8.00	8.00	6.00	6.00	10.0	10.0	
	Min	28.9	11.5	24.5	0.84	8.70	5.40	3.70	0.27	0.60	0.38	2.10	2.40	0.80	2.60	3.80	16.0	6.00	9.00	9.00	7.00	7.00	5.00	5.00	9.00	9.00	
R	10.9	6.8	16.9	0.25	3.50	2.10	1.60	0.04	0.08	0.09	0.09	0.90	1.00	0.80	1.20	1.00	1.00	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
A	F	3.17	6.21	4.47	2.70	3.64	2.38	2.05	0.05	0.00	0.02	2.37	1.44	0.00	4.62	6.09	2.45		0.96	2.26	1.90	0.16	2.45	1.30	4.36	8.18	
	dF	9	9	6	6	9	9	9	9	9	9	9	9	9	9	9	9		9	9	9	9	9	9	9	9	
	P	0.10	0.03	0.07	0.15	0.08	0.15	0.18	0.81	0.81	0.98	0.88	0.15	0.26	0.97	0.06	0.03	0.15		0.35	0.16	0.20	0.69	0.15	0.28	0.06	0.01
	DM	3.47	2.62	7.27	0.10	1.02	0.61	0.43	0.00	0.00	0.00	0.00	0.25	0.21	0.00	0.32	0.47	0.42	6.42	0.32	0.46	0.25	0.10	0.42	0.28	0.57	0.71
	DD	M > F	M > F	M > F	M > F	M > F	M > F	M > F	M > F	M = F	M = F	M = F	M > F	M > F	F=M	M > F	M > F	M > F	M > F	M < F	M > F	M > F	M < F	M < F	M < F	M < F	M < F

Table 2. Comparison three new *Hemidactylus* species with other *Hemidactylus* which occur in Iran. Data from: (1): Carranza and Arnold 2012; (2): Torki et al. 2011; (3): FTHM collections. Abbreviation are as given in Materials and Methods and Table 1 header.

Characters	<i>H. achaemenidicus</i> sp.n.	<i>H. sassanidianus</i> sp.n.	<i>H. pseudoromeshkanicus</i> sp.n.	<i>H. persicus</i> (1)	<i>H. romeshkanicus</i> (2)	<i>H. flaviviridis</i> (3)
SVL	28–39	48–63	74–75	36–67	70	59–79
TL	24–41	66–79	88	55–77	83	60–97
SL	9–10	10–13	11	10–13	15	12–17
IL	7–8	8–10	9	8–11	9	11–12
HL	8.7–12.2	13.5–20.6	22.8–23.4	9.1–16.8	23.2	17–23
HW	5.4–7.5	9.7–13	14.6–15.2	7.1–14.4	14.5	12.5–18.5
HH	3.7–5.3	6.1–8.9	8.7–9.1	4.9–9.6	9.1	7.9–10.9
HL/SVL	0.27–0.31	0.28–0.31	0.30–0.31	0.21–0.28	0.33	0.28–0.31
HW/HL	0.60–0.68	0.60–0.71	0.64–0.65	0.67–0.92	0.62	0.65–0.80
HH/HL	0.39–0.47	0.35–0.45	0.37–0.40	0.42–0.60	0.39	0.40–0.48
DTR	16–17	14–16	16–17	14–16	16	–
PAP	6–8	6–8	12	8–11	12	–
LP1	5–6	6–9	11	8–9	8	8–9
LP4	9–10	9–14	15	13–14	12	11–13
PM	2	2–4	2	2	3	2

for new *Hemidactylus* species), subcaudal scales (scale like and/or enlarged vs. enlarged), less head width/head length (0.62 vs. 0.74), internarial distance (0.97 ± 0.04 vs. 1.5 ± 0.08), lower number of lamellae under the 1st pes (5.7 ± 0.1 vs. 6.1 ± 0.5), internarial distance (0.97 vs. 1.5), and nasal in contact % (0% vs. 22%) [Carranza and Arnold 2012; Šmíd et al. 2015]. Based on photograph of lectotype of *H. robustus* (Figs. 4–9 in Šmíd et al. 2015; as a female specimen), females of *H. robustus* have approximately full rugosity (lectotype of *H. robustus* is female) and it is more than in male *H. achaemenidicus* sp.n. (males of *H. achaemenidicus* sp.n. have much greater rugosity than females); dorsal tubercle density (especially on proximal part) in *H. robustus* is more than *H. achaemenidicus* sp.n. dorsal, and dorsolaterals of *H. robustus* have maximum uniformity; in contrast the dorsum of *H. achaemenidicus* sp.n. has heterogeneity of dorsal and dorsolateral tubercles; also shape and size of tubercles on dorsolateral of *H. achaemenidicus* sp.n. is different from mid-dorsum, in contrast to *H. robustus* (Fig. 4c–d); photographic comparison: limbs (especially hind limbs) in *H. achaemenidicus* sp.n. are smaller than *H. robustus*; additional differences are: longer head for *H. robustus*; smaller interlimbs for *H. robustus*; base of tail in *H. robustus* is much more flattened and in *H. achaemenidicus* sp.n. is approximately cylindrical.

Differs from *H. flaviviridis*, *H. persicus*, and *H. romeshkanicus* by smaller body size. More comparisons with *Hemidactylus* inhabiting Iran are shown in Table 2. Differs from *H. turcicus* by smaller body size (36.5 ± 0.9 mm vs. 46.0 ± 5.8 in males, 33.1 ± 2.0 mm vs. 49.2 ± 5.1 in females), short tail relative to SVL (TL 0.98 vs. 112.8% of SVL), more longitudinal tubercle rows (16.2 ± 0.1 vs. 13.8 ± 0.7), nasal in contact % (0% vs. 13.3%), 1st and 2nd postmentals in contact with 2nd infralabials (81.8% vs. 12.9%), lower number of lamellae under the 1st pes (5.7 vs. 6.6), supralabials (9.5 ± 0.1 vs. 8.3 ± 0.5), infralabials (7.8 ± 0.1 vs. 6.8 ± 0.4), number of precloacal pores (6.42 vs. 7.2), less head width/head length (0.62 vs.

0.77) [Moravec et al. 2011; Šmíd et al. 2013]. Different from *H. persicus* in body size, tail length, head shape and ratio, dorsal tubercle rows, precloacal pores, and number of lamellae under the 1st and 4th pes (see Table 2). Different from *H. romeshkanicus* in body size, tail length, head shape, precloacal pores, and number of lamellae under the 1st and 4th pes (see Table 2).

In this section *H. achaemenidicus* sp.n. is briefly compared with other *Hemidactylus* spp. from Iran. Different from *H. adensis*, *H. awashensis*, *H. lavadeserticus*, *H. mandebensis*, *H. ulii*, and *H. jumailiae* by more longitudinal tubercle rows (16.27 vs. 14, 14, 14, 13.3, 14.1, and 14) [Šmíd et al. 2013a, 2015]. Different from *H. dawudazraqi* by more dorsal tubercle rows (16–17 vs. 12–15). Different from *H. alfarraji* by precloacal pores (6–8 vs. 4) [Šmíd et al. 2016]. Different from *H. kurdicus* by postmentals (2 vs. 1) [Safaei-Mahroo et al. 2017]. Different from *H. foudaii* by precloacal pores (6–8 vs. 9) and well developed dorsal and tail tubercles (vs. less developed and protuberant dorsal and particularly tail tubercles). Different from *H. mindiae* (Jordan) and *H. asirensis* by smaller body size (36.5 mm vs. 49.3, 43–48.5 in males, 33.1 mm vs. 49.8, 38–51 in females, respectively) [Baha el Din 2005, Moravec et al. 2011; Šmíd et al. 2017]. Different from *H. saba*, *H. granosus*, *H. yerburii*, *H. montanus*, *H. minutus*, *H. homoeolepis*, and *H. mindiae* (Egypt population) by number of precloacal pores (6.42 vs. 8, 5.6, 13.7, 11.2, 5.8, 4.3, 12.8, and 4) [Baha el Din 2005; Carranza and Arnold 2012; Šmíd et al. 2013a, 2016; Vasconcelos and Carranza 2014], respectively. Different from *H. endophis* by lacking femoral pores. Different from *H. shihraensis*, *H. hajarensis*, *H. luqueorum*, *H. festivus*, and *H. alkiyumii* by smaller body size. Significantly different from *H. mindiae*, *H. lavadeserticus*, *H. dawudazraqi*, *H. shugraensis*, and *H. sinaitus* by small body size and more dorsal tubercle rows. Different from *H. leschenaultii*, *H. homoeolepis*, *H. paucituberculatus*, *H. inexpectatus*, *H. masirahensis*, and *H. lemurinus* by having large and keeled tubercles on dorsal body.



Fig. 6. Dorsal tubercles of (a) holotype and (b) paratype of *Hemidactylus sassanidianus* sp.n.

Based on recent a molecular study on *Hemidactylus* (Maximum-likelihood tree inferred using 350 bp of the 12S gene, Appendix III, by Carranza and Arnold 2012; Šmíd et al. 2013b, 2015), *H. achaemenidicus* sp.n. (FTHM005110 is the accurate specimen number) is significantly different from: *H. luqueorum*, *H. hajarensis*, *H. lemurinus*, *H. yerburi*, *H. montanus*, *H. jumailiae*, *H. alkiyumii*, *H. robustus*, *H. sinaitus*, *H. saba*, *H. shihraensis*, *H. festivus*, *H. paucituberculatus*, *H. masirahensis*, *H. inexpectatus*, and *H. homoeolepis*.

***Hemidactylus sassanidianus* sp.n.** (Figs. 6–9)
Hemidactylus persicus Torki et al. (2011)

urn:lsid:zoobank.org:act:61CDBB8A-CE1F-4219-8F66-9DB6275C577E

Holotype

ZFMK 98573, adult male, collected at the southern end of Zagros Mountains, Khaiiz, Tangestan City, Bushehr Province, Southern Iran, on 4 May 2008 (28°43'N, 51°31'E, 525 m asl).

Paratypes

ZFMK 97754–56, ZFMK 98574–77, FTHM 005029; four adult male specimens (ZFMK 97756, ZFMK 98575–77), and four adult female specimens (ZFMK

97754–55, ZFMK 98574, FTHM 005029), same data as for holotype.

Diagnosis

A small-sized *Hemidactylus*, snout-vent length at least 48.3 mm; tubercles distributed all over dorsum, except for arm; back with enlarged keeled tubercles; heterogeneity of dorsal tubercles occurred in all specimens (a few parts or most of dorsal body); dorsal scales in a few places converted into granules; granules cover snout, between eyes, upper head, neck, and in some specimens onto middle of dorsum and dorsolaterals; 2–4 postmentals; 4–8 whorls of tubercles on first half of dorsum of tail, distal part of tail without tubercles; without femoral pores; precloacal pores present; more lamellae under fingers; subcaudal scales enlarged; ventral scales not imbricate; enlarged scansors beneath fingers, scansors mostly divided, terminal scansor single; limbs without color pattern and uniform, dorsolaterals without any pattern and uniform, pattern only present on middle part of dorsum (longitudinal) of all specimens, various patterns on dorsum such as: spotty (small or large), bars (irregular and regular); ventrum without pattern.

Description of Holotype (Fig. 6)

Measurements (in mm): body size: 54.2; tail length: 79.3; interlimbs: 21.6; head width: 10.6; head length: 16.4; head depth: 6.4; eye-eye: 6.2; ear opening: 1.9; eye diameter: 4.3; forelimbs length: 18.3; hind limbs length: 24.8.

Body depressed; body, as well head are flattened; tail flattened; head triangular-shaped; two postmentals, the first postmentals are enlarged and are widely in contact together, the 2nd postmentals one behind the first enlarged postmentals, the 1st postmentals are in contact with the 1st infralabials, the 2nd postmentals are in contact with the 2nd infralabials, four scales between 2nd postmentals; infralabials: nine; supralabials: left: 11, right: 12; nostril surrounded by five scales (the 1st supralabial, rostral, internasal scale and two postlabials); nasals not in contact and separated by one small scale; ear openings are falcate-shaped, and horizontal; 14 scales between nostril and eye; 26 scales between eye and ear; 31 scales between eyes; rostrum covered by large granules and a few tubercles distributed in distal part; between eyes covered by small granules, and nine small smooth and simple tubercles distributed among them; upper head covered by smallest granules and small tubercles distributed among them; tubercles on upper ears simple and pointed; tubercles on occipital are mostly pointed; tubercles on neck are simple, pointed and keeled (heterogeneous); granules cover rostrum to neck body; tubercles distributed on dorsum, head, and limbs; tubercles extend to in front of eyes; tubercles not found on arm; most body tubercles are keeled; dorsal tubercles are keeled, a few areas of mid-dorsum covered by abnormal tubercles (heterogeneous in

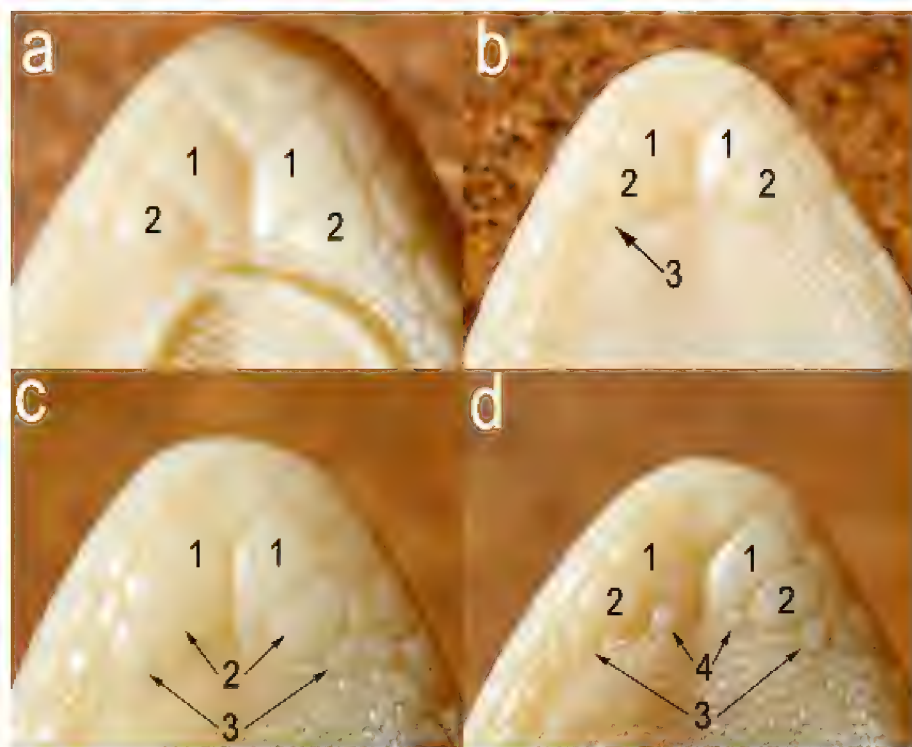


Fig. 7. Postmental variation in *Hemidactylus sassanidianus* sp.n. (a) ZFMK 98573; (b) FTHM 005029; (c) ZFMK 97756; (d) ZFMK 98575.

shape and type) and granules; dorsolateral tubercles are keeled and wide; forearm tubercles are small and simple; size of the forearm tubercles are smaller than hindlimb tubercles; number of tubercles on femur (pointed and keeled) are less than foreleg (mostly keeled); scales on palm and sole are granular; 16 regular rows of tubercles on back; 6–8 small simple tubercles between interorbits, 32 scales between interorbits (mid-part); 22 enlarged tubercles between fore- and hindlimbs; 12–14 scales surround each mid-dorsal tubercle (11–12 proximally, 12–13 distally); 3–4 scales between each dorsal tubercle.

Tail is original; 52 enlarged imbricate subcaudal scales; last part of tail cycloid-shape and covered by raised scales; proximal of tail covered by several continuous indistinct bars, 13 crossbars on dorsum of tail, tubercle whorls only found in anterior part of tail, 5–6 scales between each whorl, six tubercles in first whorl, six tubercles in the second, six in third, five in fourth, five in fifth, six in sixth, and six tubercles in the seventh whorl, after the seventh whorl tubercles become very small (six in each whorl) and converted into scales; ventral scales (mostly oval shape) are not imbricate and their size in the middle part of the body are larger than other regions; eight (4+4) precloacal pores; without femoral pores; enlarged scansors are plate-like, terminal scansor is unique (not paired); lamellae on fingers as follows: 1st: nine (1–3 undivided), 2nd: 10 (1 undivided), 3rd: 10 (1 undivided), 4th: 11, 5th: 11(1–2 undivided); lamellae on pes as follows: 1st: nine (1–2 undivided), 2nd: 11 (1 undivided), 3rd: 12 (1 undivided), 4th: 14 (1–2 undivided), 5th: 13; palm and sole covered by granule-like scales.

Coloration: upper head, neck, and middle part of dorsum covered by smallest spots and few large pale spots (background view), don't form bar; without spots or bars on dorsolaterals and limbs; one narrow stripe between nostril-eye and eye-ear; three moderate spots on snout; a pale and irregular bar on occipital and neck;

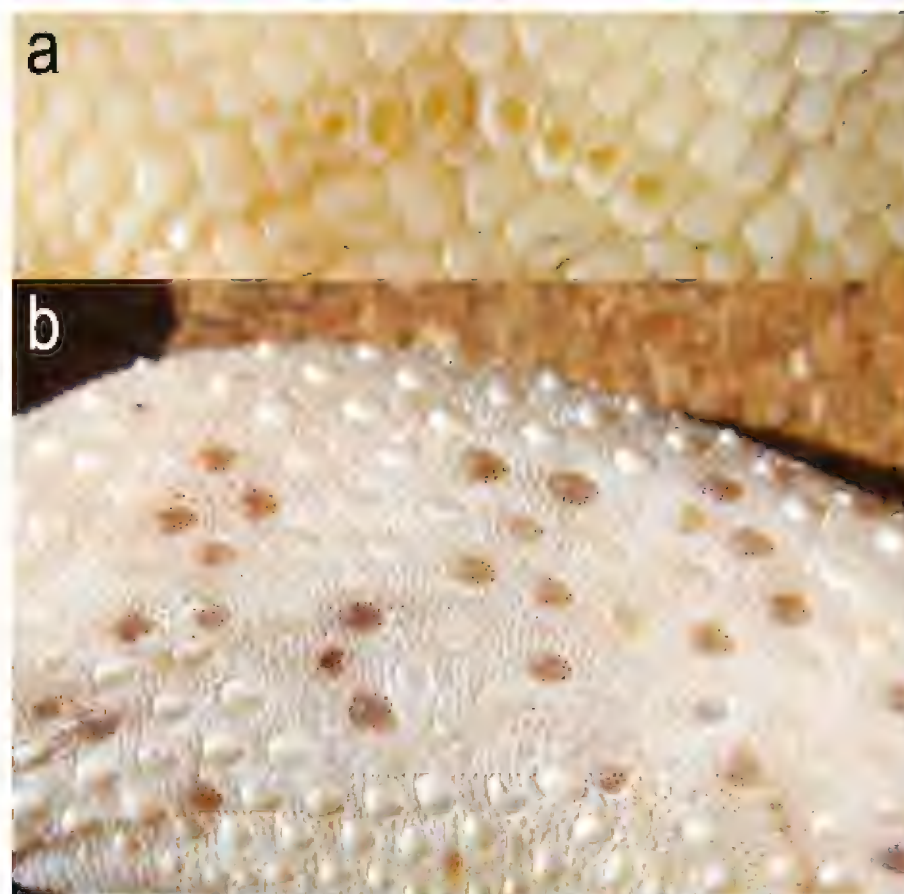


Fig. 8. (a) Precloacal pores (ZFMK 98573), and (b) dorsal tubercles (ZFMK 98573) of *Hemidactylus sassanidianus* sp.n.

color of venter is uniform white; palm of digits (hindlimbs and forelimbs) more or less white; pattern of preserved specimen is similar to the live specimen, but has lost color.

Variation (Figs. 7–8)

Heterogeneity of dorsal tubercles occurs in a few areas, mostly on the dorsal body; tubercles converted to simple (not-keeled) and have abnormal shape (e.g., rounded, width, semi), in these parts most dorsal scales converted to granules (small or large); granules cover snout and extend to upper head and neck (all specimens), or onto proximal dorsum (ZFMK 97754 and 28) or onto mid-dorsum (ZFMK 97755), lateral sides of neck strongly covered by granules and tubercles (ZFMK 97756); most specimens have 1–2 tubercles in front of ear, or 4–5 (ZFMK 98573 and ZFMK 97756) or lack tubercles (ZFMK 98577); internasals in four specimens are in contact (ZFMK 98573, ZFMK 98575, ZFMK 97754–55) and in others separated by one (ZFMK 97756, FTHM005029), two (ZFMK 98577) or three (ZFMK 98574) scales; number of postmentals is variable (usually two) between 2–4, asymmetry occurs in some of them; ZFMK 98575 have four PM as follows: 1st PM is large and in contact with 1st and 2nd infralabials, 2nd PM on posterior of 1st PM and in contact with 2nd infralabials, 3rd PM behind 2nd PM and separated from infralabials by one series of scales; 4th PM in contact with 1st and 2nd postmentals; ZFMK 97756 has three postmentals as follows: 1st larger and in contact with 1st and 2nd infralabials, 2nd PM is behind 1st PM and in contact with 2nd infralabials, 3rd PM is behind 2nd PM and separated from infralabials by one series of scales, 10 scales between 2nd postmentals; FTHM005029 has three PM on left and two on right; ZFMK 98574 has three PM on left and two on right; 4–8 whorls of tubercles on proximal half of dorsum of tail (usually six), without tubercles on distal part of tail; limbs and

Table 3. Measurements and scale counts of *Hemidactylus sassanidianus* **sp.n.** (all measurements in mm). Abbreviations: SVL: snout-vent length; TRL: trunk length; TL: tail length; R1: TL/SVL; HL: head length; HW: head width; HH: head height; R2: HL/SVL; R3: HW/HL; R4: HH/HL; OD: orbital diameter; NE: nares to eye distance; IN: internarial distance; IO1: anterior interorbital distance; IO2: posterior interorbital distance; TB: longitudinal tubercle rows; PAP: number of preanal pores; SL (L/R): number of supralabials; IL (L/R): number of infralabial scales; LP1 (L/R): number of lamellae under the first finger of the pes; LP4 (L/R): number of lamellae under the fourth finger of the pes; M: male; F: female; T: total; A: ANOVA test; F: one-way ANOVA *F* value; dF: degrees of freedom; P: probability (following Carranza and Arnold 2012); DM: difference of means; DD: direction of difference.

		SVL	TRL	TL	R1	HL	HW	HH	R2	R3	R4	OD	NE	IN	IO1	IO2	TB	PAP	SLL	SLR	ILL	ILR	LP1L	LP1R	LP4L	LP4R
	N	5	5	2	2	5	5	5	5	5	5	5	4	5	5	5	4	5	5	5	5	5	5	5	5	
	Mean	54.7	22.8	72.7	1.35	16.7	10.9	6.90	0.30	0.65	0.41	3.94	4.80	1.56	4.80	6.80	15.7	7.40	11.2	12.0	9.00	8.60	8.00	8.20	12.2	12.4
	SEM	2.44	0.87	6.55	0.10	1.13	0.58	0.52	0.00	0.01	0.01	0.32	0.24	0.16	0.26	0.27	0.25	0.40	0.37	0.31	0.31	0.40	0.54	0.58	0.86	0.92
	Max	63.3	25.9	79.3	1.46	20.6	13.0	8.90	0.31	0.71	0.45	5.00	5.20	2.20	5.70	7.80	16.0	8.00	12.0	13.0	10.0	10.0	9.00	9.00	14.0	14.0
	Min	48.3	20.8	66.2	1.25	13.5	9.70	6.10	0.28	0.61	0.37	3.20	4.10	1.30	4.20	6.20	15.0	6.00	10.0	11.0	8.00	8.00	6.00	6.00	9.00	9.00
	R	15.0	5.10	13.1	0.21	7.10	3.30	2.80	0.03	0.10	0.08	1.80	1.10	0.90	1.50	1.60	1.00	2.00	2.00	2.00	2.00	2.00	3.00	3.00	5.00	5.00
	N	4	4	1	1	4	4	4	4	4	4	4	4	4	4	4	4		4	4	4	4	4	4	4	
	Mean	56.4	26.5	67.5	1.23	17.0	10.9	6.65	0.30	0.63	0.38	3.72	5.40	1.45	4.90	6.87	15.2		12.0	12.2	8.50	8.50	9.00	9.00	13.7	13.5
	SEM	0.66	0.74			0.41	0.25	0.29	0.00	0.01	0.01	0.17	0.10	0.11	0.14	0.16	0.47		0.40	0.25	0.28	0.28	0.00	0.00	0.25	0.28
	Max	57.4	28.7	67.5	1.23	17.8	11.7	7.40	0.31	0.66	0.42	4.10	5.70	1.80	5.30	7.10	16.0		13.0	13.0	9.00	9.00	9.00	9.00	14.0	14.0
	Min	54.5	25.4	67.5	1.23	15.9	10.6	6.10	0.29	0.60	0.35	3.30	5.20	1.30	4.60	6.40	14.0		11.0	12.0	8.00	8.00	9.00	9.00	13.0	13.0
	R	2.9	3.3	0.00	0.00	1.90	1.10	1.30	0.02	0.06	0.07	0.80	0.10	0.50	0.70	0.70	2.00		2.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00
	N	9	9	3	3	9	9	9	9	9	9	9	8	9	9	9	8	5	9	9	9	9	9	9	9	9
	Mean	55.4	24.4	71.0	1.31	16.8	10.9	6.78	0.30	0.64	0.40	3.84	5.10	1.51	4.84	6.83	15.5	7.40	11.5	12.1	8.77	8.55	8.44	8.55	12.8	12.8
	SEM	1.35	0.85	4.16	0.07	0.62	0.32	0.30	0.00	0.01	0.01	0.18	0.16	0.10	0.15	0.16	0.26	0.40	0.29	0.20	0.22	0.24	0.33	0.33	0.53	0.53
	Max	63.3	28.7	79.3	1.46	20.6	13.0	8.90	0.31	0.71	0.45	5.00	5.70	2.20	5.70	7.80	16.0	8.00	13.0	13.0	10.0	10.0	9.00	9.00	14.0	14.0
	Min	48.3	20.8	66.2	1.23	13.5	9.70	6.10	0.28	0.60	0.35	3.20	4.10	1.30	4.20	6.20	14.0	6.00	10.0	11.0	8.00	8.00	6.00	6.00	9.00	9.00
	R	15.00	7.90	13.1	0.23	7.10	3.30	2.80	0.03	0.11	0.10	1.80	1.60	0.90	1.50	1.60	2.00	2.00	3.00	2.00	2.00	2.00	3.00	3.00	5.00	5.00
	F	0.38	9.59	0.21	0.47	0.07	0.00	0.14	0.00	0.49	1.45	0.29	4.90	0.26	0.09	0.04	0.85		2.07	0.35	1.29	0.03	2.59	1.46	2.40	1.03
	dF	7	7	1	1	7	7	7	7	7	7	7	6	7	7	7	6		7	7	7	7	7	7	7	7
	P	0.55	0.01	0.72	0.61	0.78	0.94	0.71	1.00	0.50	0.26	0.60	0.06	0.62	0.77	0.83	0.39		0.19	0.57	0.29	0.85	0.15	0.26	0.16	0.34
	DM	1.75	3.68	5.25	0.12	0.37	0.05	0.25	0.00	0.01	0.02	0.21	0.60	0.11	0.10	0.07	0.50	7.40	0.80	0.25	0.50	0.10	1.00	0.80	1.55	1.10
	DD	M < F	M < F	M > F	M > F	M < F	M = F	M > F	M = F	M > F	M > F	M > F	M < F	M > F	M < F	M < F	M > F	M > F	M < F	M < F	M > F	M > F	M < F	M < F	M < F	M < F



Fig. 9. Type locality of *Hemidactylus sassanidianus* sp.n., Tangestan, Bushehr, southern Iran.

dorsolaterals are uniformly without pattern, pattern only present on middle part of dorsum (longitudinal) of all specimens, various patterns are visible on dorsum such as: spots (small or large), bars (irregular and regular); ZFMK 98574: 1st bar is full and wide, 2nd as well as 3rd are X-shaped, and usually 4th bar as well; ZFMK 98575: narrow longitudinal stripe on middle part of dorsum, two bars (usually X-shaped) on neck; FTHM005029: six bars on dorsum, five regular and one irregular, one irregular bar on neck; FTHM005029: one longitudinal stripe from neck to tail; upper head and between eyes of most specimens covered by small spots. More data on the variations are shown in Table 3.

Sexual dimorphism is evident. In general, 12 characters are larger in females and 11 characters larger in males. Females (26.5 ± 0.74) have significantly ($P = 0.01$, $f = 9.59$) larger trunk length than males (22.8 ± 0.87), as the result of fecundity selection (e.g., Andersson 1994; Torki 2012), and females have a larger trunk for the development of two large eggs. The lamellae in females (1st: 9; 4th: 13.7) number more than in males (1st: 8; 4th: 12.2), which may be the result of natural selection, because during development of the two large eggs females must have more ability to move (Torki 2012); also, females have minimal variability of number of lamellae (1st: 0; 4th: 1) in contrast to males (1st: 3; 4th: 5); greater number lamellae and minimal variability in females are important positive results of natural selection for survival of *H. sassanidianus* sp.n. under natural conditions (personal assumption of author). In general, all characters (except dorsal tubercles rows), especially body size (range: males: 15; females: 2.9) have more variability in males. More data on the dimorphism and variability are shown in Table 3.

Habitat and Ecology (Fig. 9)

The Tangestan region is at the end of the southern part of the Zagros Mountains, and has palm trees. *Hemidactylus sassanidianus* sp.n. is distributed in a mountainous area. This mountain is one of the Zagros Mountains and its structure is sedimentary. Shelter sites of the new *Hemidactylus* species are limited to the clefts and

caves in this mountain, with many specimens found and collected in one cave in this locality. This cave is deep—the author was able to reach a depth of more than 50 m, though the depth of this cave is said to be even more than 200 m. This cave is an important habitat for this new *Hemidactylus* and the largest population was seen only in this cave. Other species of gecko were also seen in this cave, such as *Asaccus tangestanensis*. Of the three new species described here, only *H. sassanidianus* sp.n. was seen in this cave, but *H. sassanidianus* sp.n. was not seen outside of the cave or elsewhere in the entire region. This cave probably opens into other regions, and further investigation of this cave is needed. This cave is dark during both day and night. Conditions inside the cave are moist, in contrast to the conditions outside the cave.

Distribution

Hemidactylus sassanidianus sp.n. is distributed only at the type locality, in Khaiiz, Tangestan City, Bushehr Province, southern Iran. The type locality is situated at the end of the southern Zagros Mountains, approximately 150 km from the Persian Gulf.

Sympatric Lizards and Snakes

Several lizard and snake species were observed in the type locality, including *Asaccus tangestanensis*, *Laudakia nupta*, *Trapelus agilis*, *Tropicolotes persicus*, *Coluber (sensu lato)* sp., *Macrovipera lebetina*, and *Echis carinatus*.

Etymology

The species name “*sassanidianus*” refers to “The Sasanian Empire,” also known as Sassanian, Sasanid, Sassanid or Neo-Persian Empire, which was known to its inhabitants as Ērānshahr in the Middle Persian language.

Comparisons

Hemidactylus sassanidianus sp.n. differs from *H. persicus* (based on original description by Anderson 1872) by: (1) Dorsal tubercles in *H. sassanidianus* sp.n. are not strongly keeled and in some parts tubercles are not keeled, in contrast they are strongly keeled in *H.*

Table 4. Sexual dimorphism among *Hemidactylus persicus*, *H. sassanidianus* **sp.n.**, and *H. achaemenidicus* **sp.n.** Abbreviations: HL: head length; HW: head width; HH: head height; IO1: anterior interorbital distance; IO2: posterior interorbital distance; SL: number of supralabial; IL: number of infralabial scales (all data are means). F: female, M: male, sig: significant (Carranza and Arnold 2012).

Characters	Sex	HL	HW	HH	IO1	IO2	SL	IL	HL/ SVL	HW/ HL	HH/ HL
<i>H. persicus</i>	M	14.6	11.8	7.2	5.4	7.4	11.6	9.1	0.24	0.81	0.49
	F	12.4	9.7	6	4.6	6	10.8	10	0.24	0.78	0.49
	sig.	0.03	0.02	0.03	0.03	0.01	0.03	0.01	0.49	0.46	0.87
		M > F	M > F	M > F	M > F	M > F	M > F	M < F	M = F	M > F	M = F
<i>H. sassanidianus</i> sp.n.	M	16.7	10.9	6.9	4.8	6.8	11.6	8.8	0.30	0.65	0.41
	F	17.0	10.9	6.6	4.9	6.8	12.1	8.5	0.30	0.63	0.38
	sig.	0.78	0.94	0.71	0.77	0.83	0.19	0.29	1.00	0.50	0.26
		M < F	M = F	M = F	M < F	M = F	M < F	M > F	M = F	M > F	M > F
<i>H. achaemenidicus</i> sp.n.	M	10.9	6.91	4.68	3.32	4.70	9.57	7.92	0.29	0.62	0.42
	F	9.95	6.30	4.25	2.90	4.22	9.50	7.75	0.29	0.62	0.42
	sig.	0.08	0.15	0.18	0.06	0.03	0.80	0.24	0.81	0.98	0.88
		M > F	M > F	M > F	M > F	M > F	M > F	M > F	M = F	M = F	M = F

persicus. (2) Heterogeneity of dorsal tubercles occurred in all specimens of *H. sassanidianus* **sp.n.**, in contrast to original description of *H. persicus*. (3) Size of tubercles in *H. sassanidianus* **sp.n.** is smaller than *H. persicus*, about 0.4 of ear opening vs. 0.5 ear opening. (4) Five or six tubercles in each row of the tail in *H. sassanidianus* **sp.n.**, and in contrast *H. persicus* have seven tubercles in each row of the tail. (5) Dorsal body of *H. sassanidianus* **sp.n.** covered by spots (not bars), in contrast dorsal body in *H. persicus* is covered by transverse narrow band. More differences between *H. sassanidianus* **sp.n.** and *H. persicus* (based on original description and Anderson 1999): (6) *H. persicus* only has two postmentals (in all populations; there are no records in the literature), in contrast *H. sassanidianus* **sp.n.** has 2–4 postmentals. (7) In Anderson's work on *H. persicus* inhabiting Iran, he reported 9–11 preanal pores, which is clearly more than *H. sassanidianus* **sp.n.** (6–8). (8) Tail sharp in *H. sassanidianus* **sp.n.**, and not sharp in *H. persicus*. Additional differences with *H. persicus* include: number of postmentals (2–4 vs. 2), mental trihedral (vs. pentagonal); relatively fewer precloacal pores in males (6–8 vs. 9–11); number of lamellae under the first digit of the pes (6–9 vs. 8–9); body size of *H. sassanidianus* **sp.n.** males (54.7) smaller than females (56.4), this is in contrast to *H. persicus* (males: 59; females: 51.4); head longer (HL/SVL: 0.3 vs. 0.24), elongated (HW/HL: 0.64 vs. 0.8), and more flattened (HH/HL: 0.4 vs. 0.49) [Carranza and Arnold 2012]; sexual dimorphism in head size (HL, HW, and HD) occurs for *H. persicus* (males significantly larger than females), this is in contrast to *H. sassanidianus* **sp.n.**, and this is true for more characters (Table 4). Easily differentiated from *H. romeshkanicus* by number of precloacal pores (6–8 vs. 12), other differences: smaller body size, number of supralabials, and dorsal tubercle shape (not trihedral vs. enlarged trihedral). It is different from *H. robustus* by larger body size in both sexes combined (48–63 vs. 32–50) and in males (54.7 vs. 41.8) and females (56.4 vs. 43.6); more lamellae under

the 1st (8.5 vs. 6.1) and 4th (12.8 vs. 10.1) digits of the pes; more supralabials (11.8 vs. 9.4); and greater number of precloacal pores (7.4 vs. 6.1) [Carranza and Arnold 2012; Šmíd et al. 2013a, 2015] (Table 2). Different from *H. achaemenidicus* **sp.n.** by larger body size (48–63 vs. 28–39), tail, dorsal tubercle rows, number of lamellae under digits of pes, labials, and postmentals (see Table 2). Different from *H. flaviviridis* by presence of dorsal tubercles and without femoral pores. More comparisons are shown in Table 2.

In this section *H. sassanidianus* **sp.n.** is briefly compared with other *Hemidactylus* species outside of Iran. Different from *H. dawudazraqi* and *H. shihraensis* by body size (48–63 vs. 40–49 and less than 49, respectively). Different from *H. asirensis* by larger body size (48.3–63.3 mm vs. 43–48.5 in males, 54.5–57.4 mm vs. 38.3–51.1 in females) and HL/SVL (28–31% vs. 23–28%). Different from *H. alfarraji* by precloacal pores (6–8 vs. 4) [Šmíd et al. 2016]. Different from *H. kurdicus* by postmentals (2–4 vs. 1) [Safaei-Mahroo et al. 2017]. Different from *H. lavadeserticus* by enlarged keeled tubercles on back (vs. not so enlarged). Different from *H. foudaii* by precloacal pores (6–8 vs. 9) and well developed dorsal and tail tubercles (vs. less developed and protuberant dorsal, and particularly, tail tubercles). Different from *H. homoeolepis*, *H. masirahensis*, and *H. paucituberculatus* by having keeled tubercles on dorsum (vs. without tubercles on dorsum). Different from *H. inexpectatus*, *H. endophis*, *H. hajarensis*, *H. yerburii*, *H. shugraensis*, *H. yerburii yerburii*, *H. montanus*, *H. awashensis*, *H. minutus*, *H. homoeolepis*, *H. mindiae*, *H. lemurinus*, and *H. granosus* by number of precloacal pores (6–8 vs. 4, 14, 4–6, 12.8, 5, 13.7, 11.2, 4.5, 5.8, 4.3, 4, 6, 5.6, respectively) [Šmíd et al. 2013a, 2015, 2016; Vasconcelos and Carranza 2014; Carranza and Arnold 2012]. Different from *H. luqueorum* and *H. homoeolepis* by body size (55.4 vs. 76.8, 31.8) [Carranza and Arnold 2012]. Different from *H. turcicus* by postmentals (2–4 vs. 2), more longitudinal tubercles on dorsum (15.5 vs.



Fig. 10. Dorsal and ventral view of (a, b) holotype and (c, d) paratype specimens of *H. pseudoromeshkanicus* sp.n.

13.8), more lamellae under the 1st (8.5 vs. 6.5) and 4th (12.8 vs. 9.7) digits of the pes, and more supralabials (11.8 vs. 8.2) and infralabials (8.6 vs. 6.7) [Šmíd et al. 2013a]. Different from *H. sinaitus* by larger body size in males (54.7 vs. 39.5) and females (56.4 vs. 45.6), more lamellae under the 1st (8.5 vs. 5.7) and 4th (12.8 vs. 9.7) digits of the pes, and more supralabials (11.8 vs. 8.7) [Carranza and Arnold 2012]. Different from *H. jumailiae* by more supralabials (11.8 vs. 9.8), more lamellae under the 1st (8.5 vs. 6.9) and 4th (12.8 vs. 10.9) digits of the pes (Šmíd et al. 2013a). Different from *H. festivus*, *H. alkiyumii*, and *H. saba* by more longitudinal tubercles on dorsum (15.5 vs. 13.3, 12.9, 14) [Šmíd et al. 2013a; Carranza and Arnold 2012]. Different from *H. ulii*, *H. mandebensis*, and *H. adensis* by larger body size in males (54.7 vs. 38.6, 41.5, 34) and females (56.4 vs. 40.1, 35, 36.7), and more longitudinal tubercles on dorsum (15.5 vs. 14.1, 13.3,

14) [Šmíd et al. 2013]. Different from *H. lemuringus*, *H. masirahensis*, *H. inexpectatus*, *H. paucituberculatus*, *H. homoeolepis*, *H. leschenaultii*, and *H. flaviviridis* by having numerous enlarged tubercles on upper surface of body (vs. no enlarged tubercles on upper surface of body).

***Hemidactylus pseudoromeshkanicus* sp.n.** (Figs. 10–12)

urn:lsid:zoobank.org:act:ACECB18C-9C39-4270-A404-BCD88DFCAA52

Holotype

ZFMK 98578, adult male, collected on the western slope of central Zagros Mountains, Kole-Saat region Andimeshk, Khuzestan Province, western Iran on 14 June 2010 (32°52'N, 48°43'E, altitude 607 m asl) by Farhang Torki.

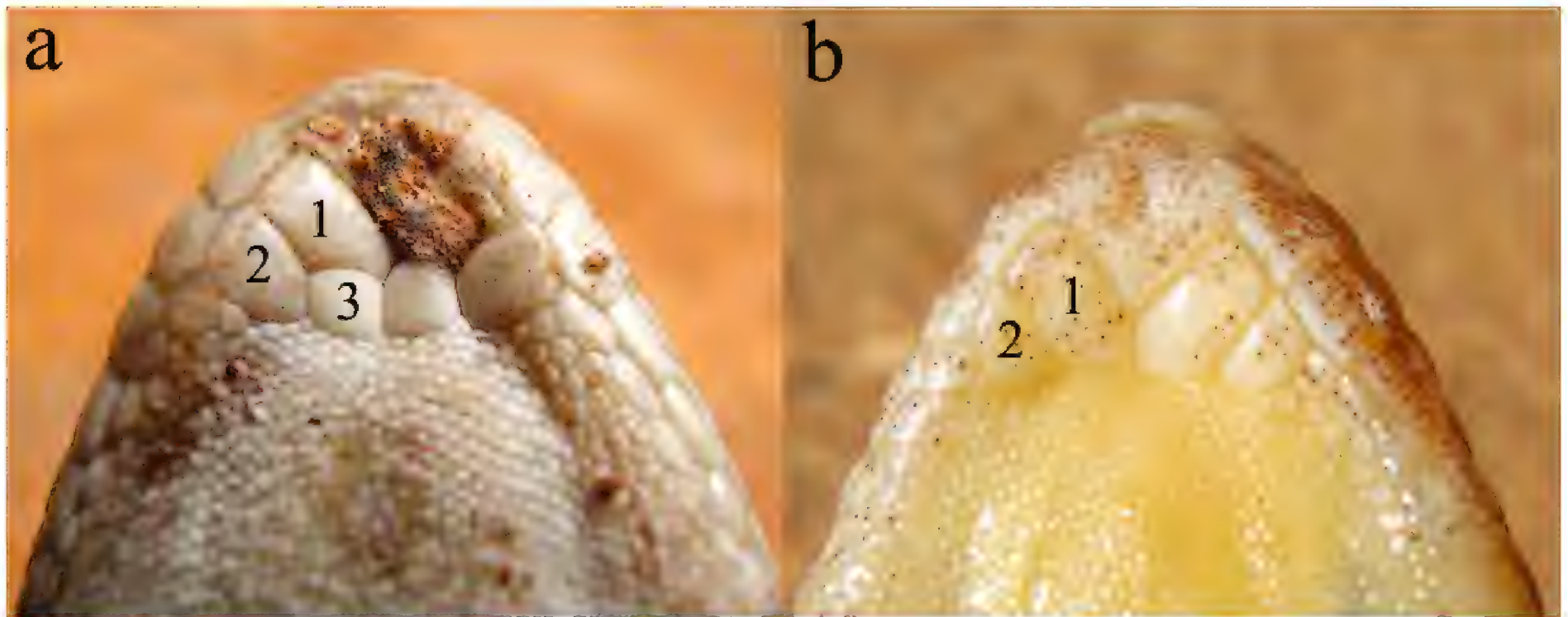


Fig. 11. Comparison of postmentals (PM). **(a)** Three well developed PM in *H. romeshkanicus* (Holotype, ZMB 75020) and **(b)** two postmentals of *H. pseudoromeshkanicus* sp.n. (Paratype, ZFMK 97757).

Paratype

ZFMK 97757, adult female, same data as for holotype.

Diagnosis

A medium sized *Hemidactylus*, snout-vent length at least 74 mm; tubercles distributed all over the dorsum (except for arms); back with enlarged trihedral keeled tubercles; granules (rather than scales) cover head and extend to neck, and rarely to forelimbs; without femoral pores; precloacal pores present; tubercular heterogeneity present on dorsum (proximal and distal parts), limbs, neck, head, and dorsolateral; six tubercles in all whorls of tail; two postmentals; more lamellae under fingers; subcaudal scale enlarged; ventral scales not imbricate, and the ends of ventral scales are simple (cycloid at mid-part; weakly denticulate at distal and proximal parts of ventral); enlarged scansors beneath fingers, scansors are mostly divided, terminal scansor is single; intermixed color pattern on dorsal body; sexual dichromatism (in both dorsal and ventral body) occurs between male (holotype) and female (paratype).

Description of Holotype (Fig. 10a–b)

Measurements (in mm): body size: 75.2; tail length: 88.7; interlimbs: 30.8; head width: 15.2; head length: 23.4; head depth: 8.7; eye-eye: 8.8; ear opening: 3.2; eye diameter: 5.4; forelimbs length: 29.9; hind limbs length: 33.3.

Body depressed; body, as well as head flattened; tail more or less flattened; head triangular-shaped; two postmentals, the first postmentals are enlarged and are in contact, the 2nd postmentals behind the 1st enlarged postmentals; the 1st postmentals are in contact with the 1st infralabials, the 2nd postmentals are in contact with the 1st and the 2nd infralabials, nine scales between 2nd postmentals; infralabials: nine; supralabials: 11; nostril surrounded by five scales (the 1st infralabial, rostral, three postnasals); nasals not in contact and separated by one small scale; ear openings are falcate-shaped,

and horizontal; 19 scales between nostril and eye; 20 scales between eye and ear; rostrum covered by large granules; between eyes covered by small granules, and small tubercles (simple and rarely pointed) distributed among them; upper head covered by smallest granules and small pointed tubercles distributed among them; tubercles above ears pointed; tubercles on occipital mostly pointed and less keeled (heterogeneous); tubercles on neck pointed and keeled (heterogeneous); from rostrum to neck covered by granules; tubercles distributed on dorsum, head, and limbs; tubercles not found on arms; most body tubercles are keeled; dorsal tubercles are enlarged, mostly trihedral and strongly keeled, some of them pointed especially between limbs (cross view); keeled tubercles between hindlimbs (cross view: proximal dorsum) intermixed with small and moderate pointed tubercles, tubercles heterogeneous (small, large, keeled, pointed, simple) obvious on distal dorsum (near tail); tubercles on femur mostly trihedral and keeled (mostly scale-like, different from tubercles on dorsum); tubercles on forearm are keeled (scale-shape, different shape from tubercles on dorsum), pointed and simple (heterogeneous in size and shape); size of the forearm tubercles smaller than hindlimb tubercles; scales on palm and sole are granule-like; 16 regular rows of tubercles on back; 11–13 small tubercles (simple or pointed) between interorbits; 23 enlarged interlimb tubercles; 16–17 scales surround each dorsal tubercle; 4–5 scales between dorsal tubercles; tail is original; 53 enlarged imbricate scales on subcaudal; last part of tail cycloid-shape and covered by raised scales; 22 crossbars on dorsum of tail, 1–3 crossbars are irregular and other crossbars are regular; tubercle whorls only found in first part of tail, five scales between each whorl, six tubercles in 1st whorl, six tubercles in the 2nd, six tubercles in 3rd, and six tubercles in the 4th whorl, after the 5th whorl tubercles become very small (six in each whorl); ventral scales are not imbricate and their size at mid-body are larger than in other regions, the ends of ventral scales are



Fig. 12. Type locality of *H. pseudoromeshkanicus* sp.n. in Kole-Saat, Andimeshk, Khuzestan province.

simple (mostly cycloid, not denticulate); 12 precloacal pores; without any femoral pores; enlarged scansors are plate-like, terminal scansor is unique (not paired); 1st scansor in most fingers is unique; lamellae on fingers as follows: 1st: 11 (1–3 undivided), 2nd: 11, 3rd: 12, 4th: 13, 5th: 13 (1–3 undivided); lamellae on pes as follows: 1st: 11 (1–3 undivided), 2nd: 12, 3rd: 13, 4th: 15, 5th: 15; claws in front of scansors; palm and sole covered by granule-like scales.

Coloration: irregular grayish pattern covers most of dorsum extending onto dorsolaterals; occipital covered by one spotted-bar that extends into eyes; snout is light grayish; neck region covered by one great grayish spotted-bar; forearm covered by small gray spots; hindlimbs covered by light irregular bars that are in contact with one another; proximal tail covered by irregular bars that are in contact together, black bars cover distal tail; arm is without spots; dorsal view of hindlimb digits darker than forelimb digits; chin is yellowish and light red; color of ventrum more or less yellowish, without any spots or bars; palms of digits (hindlimbs and forelimbs) are ashy. Pattern is similar to the live specimen and all spots and bars are obvious in preserved specimens; the preserved specimen is colorless.

Description of Paratype (Figs. 2c–d, 11b)

Measurements (in mm): body size: 74.2; tail length: not original; interlimbs: 31.7; head width: 14.6; head length: 22.8; head depth: 9.1; eye-eye: 9.8; ear opening: 3.2; eye diameter: 5.1; forelimbs length: 27.4; hind limbs length: 32.4.

Most data are similar to holotype, but some small differences as follows: 11 scales between 2nd postmentals; between eyes covered by small granules, and small tubercles (simple, pointed, and rarely keeled) distributed among them; upper head covered by smallest granules and small pointed (rarely keeled) tubercles distributed among them; tubercles on neck are less pointed and mostly keeled (heterogeneous); 17 regular rows of tubercles on back; 18 enlarged tubercles between fore- and hindlimbs; 16–18 scales surround each dorsum

tubercle; tail is missing (most part), zigzag form (without any crossbars), tubercle whorls only found in first part of tail, six tubercles in all whorls, 6–7 scales between each whorl, whorl tubercles distinct by 1–3 scales; ventral scales are not imbricate and their sizes at mid-body are larger than in other regions, the ends of ventral scales are simple (cycloid at mid-part; weakly denticulate at distal and proximal parts of ventral); without precloacal pores; without any femoral pores; enlarged scansors are plate-like, terminal scansor is unique (not paired); first scansors of most fingers are unique; lamellae on fingers as follows: 1st and 2nd: 11, 3rd: 12, 4th and 5th: 13; lamellae on pes as follows: 1st: 11, 2nd: 13, 3rd: 14, 4th and 5th: 15.

Color pattern: intermixed irregular (in contact) black and grayish pattern covers most parts of dorsum that extend onto dorsolaterals; bar and inter-bar cover proximal and distal dorsum; an irregular black stripe extends to eyes; neck region covered by one great black bar; one narrow black stripe between eyes and nostrils; one wide black stripe between eye and ear which extends to occipital region; forearm covered by small gray spots, hindlimbs covered by irregular bars that are in contact with one another; tail covered by irregular bars that are in contact (without crossbar on tail); arm is without spots; in dorsal view hindlimb digits strongly darker than forelimb digits; chin is yellowish, color of ventrum is light, without any spots or bars; palms of digits (hindlimbs and forelimbs) are white or less ashy. Pattern is similar to the live specimen and all spots and bands are obvious in preserved specimen. The preserved specimen is colorless.

Habitat and Ecology (Fig. 12)

Specimens belonging to *Hemidactylus pseudoromeshkanicus* sp.n. were collected from the Kol-e-Saat region, Andimeshk, Khuzestan province. Kol-e-Saat Region is located between Lorestan-Khuzestan Provinces and has warm climatic conditions; it is located between the central Zagros Mountains and Khuzestan Plain. Oak (*Quercus brantii*) forest is distributed in the mountains of this region. The new *Hemidactylus* specimens show nocturnal activity, and feed on small insects and insect larvae occurring in the habitat. Individuals of the new species actively climb on rocks, and specimens were collected on rocks during the middle of the night.

Distribution

Presently, this new species is only recorded from the type locality at Kol-e-Saat region, Andimeshk, Khuzestan Province, Iran. In spite of several field trips to areas adjacent to the type locality, no specimens belonging to this new taxon were found. But based on geomorphological patterns of the folded mountains of the western slope of Zagros Mountains, the main distribution of *H. pseudoromeshkanicus* sp.n. is expected to extend towards the mountains of northern Khuzestan province.

Sympatric Lizards and Snakes

From the type locality the following additional reptile species were recorded: *Asaccus nasrullahi*, *Cyrtopodion scabrum*, and *Pseudocerastes fieldi*.

Etymology

The name “*pseudoromeshkanicus*” is an allusion to its similarity to *H. romeshkanicus*. The color pattern of this new species appears similar to *H. romeshkanicus*, but morphological characters do not match this species, therefore the prefix “*pseudo*” is used for the new species.

Comparison with *Hemidactylus romeshkanicus*

Hemidactylus pseudoromeshkanicus **sp.n.** is significantly different from *H. romeshkanicus* by several characters as follows: two postmentals (instead of three developed in *H. romeshkanicus*, Fig. 11); *H. pseudoromeshkanicus* **sp.n.** has more lamellae under 4th digit of pes (13 instead of nine), 1st digit (11 instead of eight), and 4th digit (15 instead of 12) than *H. romeshkanicus* (which is slightly true for other fingers); whorl tubercles on tail (number, size, and arrangement) as follows: number of tubercles in each whorl in *H. pseudoromeshkanicus* **sp.n.** from 1st to 4th is unique (6-6-6-6), in contrast in *H. romeshkanicus* decreasing number of tubercles from 1st to 4th whorl (7-6-5-4), scales between each whorl in *H. pseudoromeshkanicus* **sp.n.** more than *H. romeshkanicus* (5–7 instead of four); supralabials in *H. pseudoromeshkanicus* **sp.n.** significantly less than *H. romeshkanicus* (11 instead of 15); tubercle rugosity (in general) on dorsum of body of *H. romeshkanicus* is stronger than *H. pseudoromeshkanicus* **sp.n.** (one significant example: three views of trihedral tubercles show rugosity, that rarely occurs for *H. pseudoromeshkanicus* **sp.n.**), tubercular heterogeneity (small and large trihedral, pointed) occurs on proximal and distal part of dorsum of *H. pseudoromeshkanicus* **sp.n.**, in contrast to *H. romeshkanicus*. Nasals separated by one small scale in *H. pseudoromeshkanicus* **sp.n.**, in contrast, one large scale separates nasals in *H. romeshkanicus*.

Comparisons with other *Hemidactylus*

In general, *H. pseudoromeshkanicus* **sp.n.** is significantly different from *H. robustus*, *H. persicus*, *H. sassanidianus* **sp.n.**, and *H. achaemenidicus* **sp.n.** by having mostly enlarged trihedral tubercles on dorsal body. Differs from *H. robustus* in body size (than less 50 vs. at least 74 mm) and tail with more precloacal pores (12 vs. 6–8), tail tuberculation (keeled and raised instead pointed), and different dorsal color patterns (irregular bands vs. spotted). Differs from *H. persicus* by larger body size and stronger tubercle rugosity on entire dorsal body and limbs, head shape and size, and dorsal tubercle rows (Table 2). Differs from *H. flaviviridis* by having enlarged tubercles on dorsum, and without femoral pores. For more comparisons see Table 2. Differs from *H. sassanidianus* **sp.n.** and *H. achaemenidicus* **sp.n.** by having more

precloacal pores (12 vs. 6–8, 6–8, respectively), larger body size, tail with more dorsal tubercle rows, dorsal tubercle shape and size (more rugosity and larger in size for *H. pseudoromeshkanicus* **sp.n.**), and more lamellae under fingers (Table 2).

Brief comparisons show differences of *H. pseudoromeshkanicus* **sp.n.** from other *Hemidactylus* spp. outside of Iran. Differs from *H. dawudazraqi*, *H. hajarensis*, *H. homoeolepis*, *H. jumailiae*, *H. shihraensis*, *H. alfarraji*, *H. asirensis*, and *H. foudaii* by precloacal pores (12 vs. 6–8, 4–6, 3–6, 6–9, 6, 4, 6, 8–10, respectively). Differs from *H. kurdicus* by postmentals (2 vs. 1) and precloacal pores (12 vs. 10) [Safaei-Mahroo et al. 2017]. Differs from *H. montanus* by more lamellae beneath 4th digit of pes (15 vs. 9–12). Differs from *H. endophis* by large body size (74–75 vs. 59), strongly keeled dorsal tubercles (vs. relatively weakly keeled), and without femoral pores (vs. 14 pores). Differs from *H. lemurinus* by presence of well-developed dorsal tubercles (vs. none). Differs from *H. luqueorum*, *H. festivus*, *H. paucituberculatus*, *H. lavadeserticus*, *H. masirahensis*, and *H. inexpectatus* by more precloacal pores (12 vs. 5–6, 6, 6, 6, 4, and 4, respectively). Differs from *H. turcicus* by larger body size and tail, more lamellae beneath 4th digit of pes (13 vs. 8–11), more precloacal pores (12 vs. 6–10), stronger tubercular rugosity, and different body color patterns. Differs from *H. mindiae*, *H. granosus*, *H. mandebensis*, *H. awashensis*, *H. adensis*, *H. minutus*, *H. ulii*, *H. saba*, *H. jumailiae*, and *H. yerburii*, by having larger body size. Differs from *H. alkiyumii* by having more rows of tubercles (16–17 vs. 11–14), more lamellae under the 4th digit of pes (15 vs. 10–12), and more precloacal pores (12 vs. 6–10). Body size in *H. pseudoromeshkanicus* **sp.n.** is smaller than in *H. aaronbaueri*, dorsal tubercles in *H. pseudoromeshkanicus* **sp.n.** are much larger than in *H. aaronbaueri*; also, color pattern is different from *H. aaronbaueri*. By having enlarged, trihedral, and regular dorsal tubercles *H. pseudoromeshkanicus* **sp.n.** is easily distinguished from several species of *Hemidactylus* including: *H. aaronbaueri*, *H. bowringii*, *H. brookii*, *H. flaviviridis*, *H. garnotii*, *H. karenorum*, *H. leschenaultii*, *H. maculatus*, *H. persicus*, *H. prashad*, *H. subtriedrus*, and *H. triedrus*. Digits are relatively slender in *H. scabriceps*, but in *H. pseudoromeshkanicus* **sp.n.** they are broadly dilated. *H. sinaitus* (from Sudan to Northern Somalia, and Arabia) has smaller and more widely separated dorsal tubercles, but *H. pseudoromeshkanicus* **sp.n.** has mostly trihedral tubercles.

Note on *Hemidactylus* Inhabitants from Iran

Hemidactylus inhabitants of the Iranian plateau have a complicated history. Anderson (1999) reported three *Hemidactylus* (*H. flaviviridis*, *H. persicus*, and *H. turcicus*) from Iran. Anderson (1974) had recorded *H. garnotii* in the fauna of Iran, but in 1999 he excluded

it from Iran due to incomplete data from I. Darevsky; and he then diagnosed this species as *H. flaviviridis* (Anderson 1999). Anderson collected some *Hemidactylus* sp. specimens from southwest Iran that do not match *H. flaviviridis*, *H. persicus*, or *H. turcicus* (Anderson 1999). Anderson was concerned that *H. brookii* might be distributed in southern Iran, but this species has not been collected inside Iran. Therefore, based on Anderson's studies (1999), four species occur in Iran: *H. flaviviridis*, *H. persicus*, *H. turcicus*, and *Hemidactylus* sp. A molecular study (Bauer et al. 2006a) confirmed the distribution of *H. robustus* in southwestern Iran; and, little difference exists between *H. robustus* from Iran on the one hand and from the United Arab Emirates and Egypt on the other. Firouz (2000) has cited *H. flaviviridis*, *H. persicus*, and *H. turcicus* for the fauna of Iran. Torki et al. (2011) showed five *Hemidactylus* species to occur in Iran, viz: *H. flaviviridis*, *H. persicus*, *H. turcicus*, *H. robustus*, and *H. romeshkanicus*. Due to this author's revision of the gecko fauna of Iran (2016–2020 FTE program), one previous occurrence of *Hemidactylus* was identified as *H. turcicus* (FTHM005100–5110 in Torki et al. 2011); however, new morphological evidence shows that it is completely different from *H. turcicus* as well as from *H. robustus*. As described here, this population (*H. achaemenidicus* sp.n.) shows differences in important taxonomic characters from other *Hemidactylus* species both inside and outside of Iran (as well as the arid clade). Hosseinzadeh et al. (2014) worked on the morphology of *Hemidactylus* species of Iran, and their work showed four *Hemidactylus* species from Iran: *H. flaviviridis*, *H. persicus*, *H. robustus*, and *H. romeshkanicus*, as they rejected *H. turcicus* from the Iranian gecko fauna. Based on recent phylogenetic studies on *Hemidactylus*, particularly *H. turcicus* and *H. robustus* (Carranza and Arnold 2012; Šmíd et al. 2013b, 2015), I suggest that the *H. robustus* specimens which were examined by Hosseinzadeh et al. (2014) do match with both *H. turcicus* and *H. robustus*. They do not show the important taxonomical characters that are important for diagnosis of *H. turcicus* and *H. robustus* from several of those populations.

Based on recent molecular studies (Carranza and Arnold 2012; Šmíd et al. 2013b, 2015), *H. persicus* from Iran shows characteristics of being a separate clade from Arabian *Hemidactylus*. This clade shows three distinguishable species, and one of them (FTHM005110) is the new *Hemidactylus achaemenidicus* sp.n. described here. The locality of FTHM005110 cited in that phylogenetic study is incorrect and must be changed to the type locality of *H. achaemenidicus* sp.n. given here. On the other hand, three specimens of *H. persicus* (JS103–5) among the Iranian *persicus* clade (Šmíd et al. 2013) showed more differences from other *H. persicus*, but the localities of these specimens were not cited in that paper, and are nearest to the type locality of *H. sassanidianus* sp.n. (see Fig. 4 in Šmíd et al. 2013). On the other hand,

H. robustus from the coastal Persian Gulf (Bandar-e-Lenge) is a match with the Arabian *H. robustus* clade (Šmíd et al. 2013b, 2015). The oldest reported dispersal from Arabia occurred 13.1 Ma, when the ancestor of *H. persicus* colonized Iran (Šmíd et al. 2013b). This time-frame (13.1 Ma) is perfect for speciation among the *Hemidactylus* inhabiting the Iranian plateau as well as the Zagros Fold-Thrust Belt. A few collections from the southern part of Iran (mostly coastal Persian Gulf) show three clades in the phylogenetic tree of Šmíd et al. (2013b). Based on the distribution of *Hemidactylus* inside the Iranian plateau, here I suggest that *Hemidactylus* has several monophyletic clades as well as more species which remain unknown.

Although some works **exclude** *H. turcicus* (e.g., Hosseinzadeh et al. 2014, Šmíd et al. 2014) from the fauna of Iran, Šmíd et al. (2014) did not explicitly **reject** *H. turcicus* from Iran (see Map 46), and Šmíd et al. concluded that *H. turcicus* is not distributed in Iran. I disagree with those assessments, and do not exclude this widespread species from the fauna of Iran until more comprehensive data about the *Hemidactylus* inhabiting Iran (especially from phylogenetic studies) are available. One important reason supporting the acceptability of *H. turcicus* for the fauna of the Iranian Plateau is its wide distribution in adjacent areas to the west (e.g., Turkey) and east (e.g., Pakistan) of Iran (e.g., Turgay and Atat 1994; Khan 2006).

Bauer et al. (2006a) identified all populations of small *Hemidactylus* as a *H. robustus*. Some authors (e.g., Gholamifard and Rastegar-Pouyani 2011; Hosseinzadeh et al. 2014) followed that assessment. Based on phylogenetic analysis, *H. achaemenidicus* sp.n. is completely distinguishable from *H. robustus* (e.g., Šmíd et al. 2013, 2015). Therefore, there are at least three distinct species of small *Hemidactylus* in Iran including: *H. robustus*, *H. turcicus*, and *H. achaemenidicus* sp.n.

Based on all the studies cited above, all *Hemidactylus* species of Iran (except *H. flaviviridis*) show much complexity and I classify them here in three groups as follows: *H. persicus*-complex (including *H. persicus*, *H. sassanidianus* sp.n., and *H. achaemenidicus* sp.n.); *H. robustus*-complex; and *H. romeshkanicus*-complex (*H. romeshkanicus* and *H. pseudoromeshkanicus* sp.n.).

In summary, at least eight species of *Hemidactylus* are distributed on the Iranian Plateau: *H. flaviviridis*, *H. persicus*, *H. robustus*, *H. turcicus*, *H. romeshkanicus*, *H. sassanidianus* sp.n., *H. achaemenidicus* sp.n., and *H. pseudoromeshkanicus* sp.n.

Note on *Hemidactylus parkeri* Loveridge 1936

H. parkeri was described by Loveridge (1936), but this species was downgraded or rejected from subsequent species lists of *Hemidactylus* (e.g., Arnold 1980; Šmíd et al. 2015) and replaced by *H. turcicus* and *H. robustus*. Based on the following reasons, I do not agree with

this decision. (i) Type locality: The type locality of *H. parkeri* is very far from the type localities of *H. turcicus* (Asiatic Turkey, by Moravec et al. 2011) and *H. robustus* (“Egypten, Arabien, und Abyssinien” restricted to “the Red Sea coast of the State of Eritrea” by Šmíd et al. 2015). (ii) Ecology and climate: Loveridge (1936) described his new species in Zanzibar Island (Tanzania), and this island may have an important role in the speciation of these geckos. Additionally, Zanzibar Island is located near the equator, with special ecological and climatic conditions; and the ecological and climatic conditions of the type locality of *H. parkeri* are completely different from the type localities of *H. turcicus* and *H. robustus*. (iii) New methods and insights: Based on phylogenetic studies, most *Hemidactylus* species described long ago have been split into several species, such as *H. persicus*, *H. yerburii*, *H. turcicus*, and *H. robustus* (e.g., Carranza and Arnold 2012; Šmíd et al. 2013, 2015). Therefore, additional phylogenetic studies on the equatorial *Hemidactylus* species are necessary to resolve this problem. (iv) Six species of *Hemidactylus* are distributed in Tanzania, and *H. parkeri* is not synonymous with all of them (Uetz 2019). On the other hand, only one species is endemic to Tanzania (*H. tanganicus*). Based on the above reasons, there is not a logical and scientific basis for the rejection of *H. parkeri*. Therefore, in this study I am in agreement with Lazza (1978, 1983) on the validity of *H. parkeri*.

Note on Gecko Conservation in Iran

Based on observations during 20 years, two main threats for the geckos of Iran are apparent: (i) Rumor: People in this region believe that geckos are poisonous and fear them, especially in cities and less so in villages. This rumor applies to all geckos inhabiting human homes. (ii) Trade: Among geckos, the fat-tailed gecko (*Eublepharis*) is an important species that is sold. *Eublepharis* is considered attractive and some people find it interesting as a pet. During recent years, trade of this gecko has increased among the Iranian people. Although 47% of geckos inhabiting Iran belong to the Red List, the IUCN category (<http://www.iucn.org/>) of most is LC (or Least Concern). The geckos in Iran have the best conservation situation compared to other amphibians and reptiles, and their nocturnal activity may have an important role.

Key to *Hemidactylus* Species Distributed in Iran

- 1a: Dorsal tubercles absent, femoral pores present *H. flaviviridis*
- 1b: Dorsal tubercles present, femoral pores absent....2
- 2a: 5–8 precloacal pores.....3
- 2b: 9–11 precloacal pores.....*H. persicus*
- 2c: 12 precloacal pores.....5
- 3a: 2–4 postmentals, not small *Hemidactylus* (body

size is more than 48 mm).....*H. sassanidianus* **sp.n.**
 3b: Two postmentals, small *Hemidactylus* (less than 50 mm).....4

4a: Females have less rugosity than males, subcaudals covered by small scales and/or plate-like scales (few in number: 0–22).....*H. achaemenidicus* **sp.n.**

4b: Sexual rugosity does not occur (females have approximately full rugosity), subcaudal scales enlarged (no small scales).....*H. robustus*

5a: Two postmentals.....*H. pseudoromeshkanicus* **sp.n.**

5b: Three postmentals.....*H. romeshkanicus*

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Literature Cited

- Agarwal I, Giri VB, Bauer AM. 2011. A new cryptic rock-dwelling *Hemidactylus* (Squamata: Gekkonidae) from south India. *Zootaxa* 2765: 21–37.
- Anderson J. 1872. On some Persian, Himalayan, and other reptiles. *Proceedings of the Zoological Society of London* 1872: 371–404.
- Anderson SC. 1974. Preliminary key to the turtles, lizards, and amphisbaenians of Iran. *Fieldiana Zoology* 65: 27–44.
- Anderson SC. 1999. *The Lizards of Iran*. Society for the Study of Amphibians and Reptiles, Ithaca, New York, USA. 415 p.
- Andersson M. 1994. *Sexual Selection*. Princeton University Press, Princeton, New Jersey, USA. xx + 599 p.
- Arnold EN. 1980. The scientific results of the Oman flora and fauna survey 1977 (Dhofar). The reptiles and amphibians of Dhofar, southern Arabia. *Journal of Oman Studies* Special Report 2: 273–332.
- Baha el Din SM. 2003. A new species of *Hemidactylus* from Egypt. *African Journal of Herpetology* 52: 39–47.
- Baha el Din SM. 2005. An overview of Egyptian species of *Hemidactylus* (Gekkonidae), with the description of a new species from the high mountains of South Sinai. *Zoology in the Middle East* 34: 11–26.
- Bauer AM, Jackman T, Greenbaum E, Papenfus TJ. 2006a. Confirmation of the occurrence of *Hemidactylus robustus* Heyden, 1827 (Reptilia: Gekkonidae) in Iran and Pakistan. *Zoology in the Middle East* 39: 59–62.
- Bauer AM, Tchiboza S, Pauwels OSG, Lenglet G. 2006b. A review of the gekkotan lizards of Bénin, with the description of a new species of *Hemidactylus*

- (Squamata: Gekkonidae). *Zootaxa* 1242: 1–20.
- Bauer AM, LeBreton M, Chirio L, Ineich I, Talla Kouete M. 2007. New species of *Hemidactylus* (Squamata: Gekkonidae) from Cameroon. *African Journal of Herpetology* 55: 83–93.
- Busais S, Joger U. 2011. Three new species and one new subspecies of *Hemidactylus* Oken, 1817 from Yemen (Squamata, Gekkonidae). *Vertebrate Zoology* 61(2): 267–280.
- Carranza S, Arnold EN. 2012. A review of the geckos of the genus *Hemidactylus* (Squamata: Gekkonidae) from Oman based on morphology, mitochondrial and nuclear data, with descriptions of eight new species. *Zootaxa* 3378: 1–95.
- Firouz E. 2000. *A Guide to the Fauna of Iran*. Iran University Press, Tehran, Iran. vi + 491 p.
- Gholamifard A, Rastegar-Pouyani N. 2011. Distribution of *Hemidactylus* geckos (Reptilia: Gekkonidae) in Fars Province, Southern Iran. *Amphibian & Reptile Conservation* 5(1): 1–5 (e19).
- Giri VB. 2008. A new rock dwelling *Hemidactylus* (Squamata: Gekkonidae) from Maharashtra, India. *Hamadryad* 32: 25–33.
- Giri VB, Bauer AM. 2008. A new ground-dwelling *Hemidactylus* (Squamata: Gekkonidae) from Maharashtra, with a key to the *Hemidactylus* of India. *Zootaxa* 1700: 21–34.
- Giri VB, Bauer AM, Chaturvedi N. 2003. Notes on the distribution, natural history and variation of *Hemidactylus giganteus* Stoliczks, 1871. *Hamadryad* 27(2): 217–221.
- Hosseinzadeh MS, Aliabadian M, Rastegar-Pouyani E, Rastegar-Pouyani N. 2014. Morphological study of *Hemidactylus* geckos (Squamata: Gekkonidae) from Iran. *Iranian Journal of Animal Biosystematics* 10(2): 175–184.
- Kamali K. 2013. *A Field Guide for Reptiles and Amphibians of Iran*. Iran Shenasi Publisher, Tehran, Iran. 368 p. (in Farsi)
- Khan MS. 2006. *The Amphibians and Reptiles of Pakistan*. Krieger, Malabar, Florida, USA. xvi + 311 p.
- Lanza B. 1990. Amphibians and reptiles of the Somali Democratic Republic: check list and biogeography. *Biogeographia* 14: 407–465.
- Lanza B. 1983. A list of the Somali amphibians and reptiles. *Monitore Zoologico Italiano New Series* 18(8 Supplement): 193–247.
- Loveridge A. 1936. New geckos of the genus *Hemidactylus* from Zanzibar and Manda Islands. *Proceedings of the Biological Society of Washington* 49: 59–62.
- Mahony S. 2010. Taxonomic revision of *Hemidactylus brookii* Gray: a re-examination of the type series and some Asian synonyms, and a discussion of the obscure species *Hemidactylus subtriedrus* Jerdon (Reptilia: Gekkonidae). *Zootaxa* 3042: 37–67.
- Mirza ZA, Rajesh VS. 2014. A new cryptic species of gecko of the genus *Hemidactylus* Oken, 1817 (Reptilia: Gekkonidae) from Southern India. *Taprobanica* 6(1): 12–20.
- Moravec J, Kratochvíl L, Amr ZS, Jandzik D, Šmíd J, Gvoždík V. 2011. High genetic differentiation within the *Hemidactylus turcicus* complex (Reptilia: Gekkonidae) in the Levant, with comments on the phylogeny and systematics of the genus. *Zootaxa* 2894: 21–38.
- Rastegar-Pouyani N, Johari M, Parsa H. 2006. *Field Guide to the Reptiles of Iran. Volume 1: Lizards*. 1st edition. Razi University Press, Kermanshah, Iran. (in Farsi)
- Safaei-Mahroo B, Ghaffari H, Ghafoor A, Amini S. 2017. A new species of *Hemidactylus* (Squamata: Gekkota: Gekkonidae) from Qara Dag Mountains, Kurdistan Region, with a key to the genus in Iraq. *Zootaxa* 4363(3): 377–392.
- Sindaco R, Razzetti E, Ziliani U, Wasonga V, Carugati C, Fasola M. 2007. A new species of *Hemidactylus* from Lake Turkana, Northern Kenya (Squamata: Gekkonidae). *Acta Herpetologica* 2(1): 37–48.
- Šmíd J, Moravec J, Kratochvíl L, Gvoždík V, Nasher AK, Busais SM, Wilms T, Shobrak MY, Carranza S. 2013a. Two newly recognized species of *Hemidactylus* (Squamata, Gekkonidae) from the Arabian Peninsula and Sinai, Egypt. *ZooKeys* 355: 79–107.
- Šmíd J, Carranza S, Kratochvíl L, Gvoždík V, Nasher AK, Moravec J. 2013b. Out of Arabia: A complex biogeographic history of multiple vicariance and dispersal events in the gecko genus *Hemidactylus* (Reptilia: Gekkonidae). *PLoS ONE* 8(5): e64018.
- Šmíd J, Moravec J, Kodym P, Kratochvíl L, Hosseinian S, Yousefkhani S, Rastegar-Pouyani E. 2014. Annotated checklist and distribution of the lizards of Iran. *Zootaxa* 3855: 1–97.
- Šmíd J, Moravec J, Kratochvíl L, Nasher AK, Mazuch VG, Carranza S. 2015. Multilocus phylogeny and taxonomic revision of the *Hemidactylus robustus* species group (Reptilia, Gekkonidae) with descriptions of three new species from Yemen and Ethiopia. *Systematics and Biodiversity* 13(4): 346–368.
- Šmíd J, Shobrak M, Wilms T, Joger U, Carranza S. 2016. Endemic diversification in the mountains: genetic, morphological, and geographical differentiation of the *Hemidactylus* geckos in southwestern Arabia. *Organisms, Diversity & Evolution* 17: 267–285.
- Torki F, Manthey U, Barts M. 2011. A new *Hemidactylus* Gray, 1825 from Lorestan Province, western Iran, with notes on *Hemidactylus robustus* Heyden, 1827 (Reptilia: Squamata: Gekkonidae). *Sauria* 33(4): 47–56.
- Torki F. 2012. Notes on sexual dimorphism of *Carinatogekko heteropholis* (Reptilia: Gekkonidae) (Minton, Anderson et Anderson, 1970). *Russian Journal of Herpetology* 19(4): 284–286.

Three new *Hemidactylus* species from Iran

- Turgay F, Atatr MK. 1994. Feeding biology in *Hemidactylus turcicus* (Lacertilia: Gekkonidae) populations of the Izmir Region. *Turkish Journal of Zoology* 18: 123–129.
- Uetz P. 2019. The Reptile database. Available: <http://www.reptile-database.org/> [Accessed: 10 December 2019].
- Vasconcelos R, Carranza S. 2014. Systematics and biogeography of *Hemidactylus homoeolepis* Blanford, 1881 (Squamata: Gekkonidae), with the description of a new species from Arabia. *Zootaxa* 3835(4): 501–527.
- Wagner P, Leaché AD, Fujita MK. 2014. Description of four new West African forest geckos of the *Hemidactylus fasciatus* Gray, 1842 complex, revealed by coalescent species delimitation. *Bonner Zoological Bulletin* 63(1): 1–14.



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The Blue Dyeing Poison-Dart Frog, *Dendrobates tinctorius* (*Dendrobates azureus*, Hoogmoed 1969): extant in Suriname based on a rapid survey

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Abstract.—The blue color morph of *Dendrobates tinctorius*, originally described as *D. azureus*, has only been reported from a few forest islands surrounded by the Sipaliwini savanna in Suriname, South America. The last published survey of these populations occurred in 1996. The threats of emergent diseases, illegal collecting, climate change, and habitat destruction through anthropogenic fires toward these populations are unknown. This report presents the results of a rapid survey of the three forest islands where *D. tinctorius* historically occurred to assess its current status. One 50 x 50 m survey plot was established in each of these three forest islands. A total of 23 frogs were recorded, with some individuals found in each of the forest island surveyed. These results indicate that *D. tinctorius* populations are still present at all three historical localities surveyed by previous researchers in 1968 and again in 1996, although the current surveys found fewer frogs. During the surveys there was no evidence of illegal collecting or habitat degradation. These observations provide baseline data that can be used for future monitoring and protection of one of the most geographically restricted and unique color morphs of *D. tinctorius*.

Keywords. Amphibia, Dendrobatidae, conservation, Sipaliwini savanna, population decline, habitat fragmentation, climate change

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Introduction

The Blue Dyeing Poison-dart Frog was described a half-century ago as *Dendrobates azureus* (Hoogmoed, 1969) and subsequently synonymized with *D. tinctorius* (Wollenberg et al. 2006). The inclusion of this species as a color morph of *D. tinctorius* was also confirmed by Noonan and Gaucher (2006). Ouboter and Jairam (2012) considered this species to be a subspecies of *D. tinctorius* due to the geographically isolated occurrence of this particular morph. Its classification as a subspecies was refuted by Frost (2017) who maintained this species as *D. tinctorius*. Other blue morphs of *D. tinctorius* were documented by Silverstone (1975) from Shudikar-wau, Guyana and by Avila-Pires et al. (2010) from Estação Ecológica Grão-Pará North, Brazil. However, specimens from Suriname do not display the typical light *D. tinctorius* pattern on the dorsum, whereas specimens from the other mentioned locations still have this pattern.

The predominantly blue color morph (“azureus”) found in Suriname is restricted to a few isolated “forest islands”

in the Sipaliwini savanna in southern Suriname (Hoogmoed 1969; Riezebos 1979; Cover 1997; Ouboter and Jairam 2012). This area was mapped in July 1935 when an expedition by Baron van Lynden was charged with establishing the southern border of Suriname (Lynden 1939). The Sipaliwini savanna is part of a larger savanna complex covering portions of Suriname and northern Pará, Brazil (Paru savanna). The current vegetation primarily consists of grasses, sedges, scattered shrubs, trees, and *Mauritia* palms (Oldenburger et al. 1973). The sharp demarcation between the savanna and forest islands may be due to geological factors as suggested by Hoogmoed (1973), while the anthropogenic influence of fires set during the dry season by the indigenous people might have been a reason that the savanna remained open (Oldenburger et al. 1973; Ouboter and Jairam 2012) [Fig. 1].

A number of factors potentially threaten the continued existence of these populations of *D. tinctorius* in the wild. Their extremely restricted range and small population size are factors that increase the risk of extinction (Hall et al. 2008). Anthropogenic fires could potentially reduce

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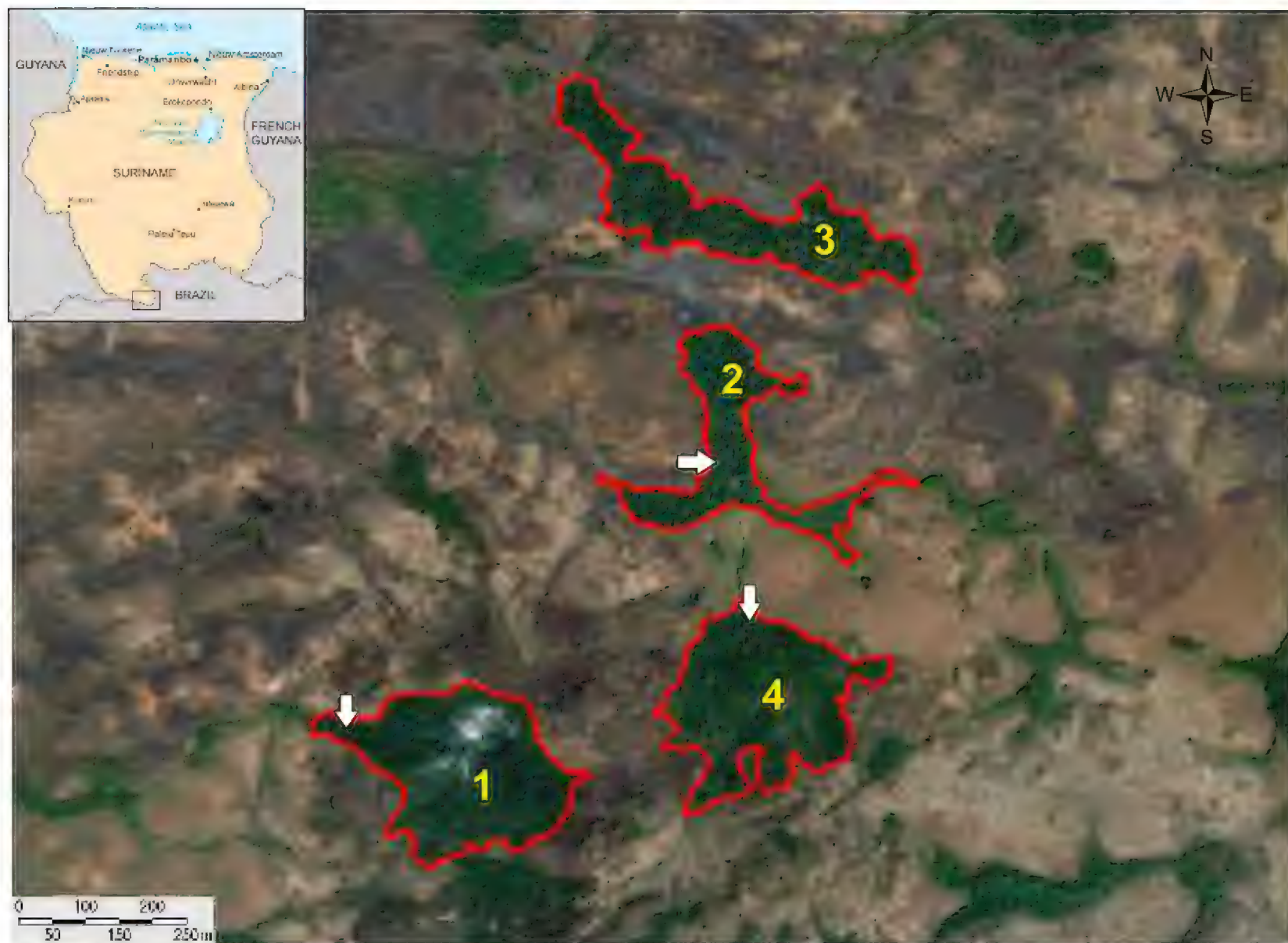


Fig. 1. Map of Suriname, South America (upper left inset) with approximate location of Sipaliwini savannah indicated by the black box. Forest Islands are outlined in red and identified by numbers corresponding to those of Hoogmoed (1969, 2019). Arrows indicate locations of the 50 x 50 m plots surveyed in Forest Islands 1, 2, and 4 during 16–18 June 2015. Forest Island 3 was not surveyed. In this 2004 Google image, savanna vegetation surrounding the forest islands had been recently burned by indigenous hunters.

the size of the forest islands, and charred trees on the periphery of the forest islands were noted by Cover (1997). The emerging infectious disease *Batrachochytrium dendrobatidis* (*Bd*) may put these populations at risk (Courtois et al. 2012, 2015; James et al. 2015). Although *Bd* has not been documented in Suriname, it has been documented in *D. tinctorius* in French Guiana (Courtois et al. 2015) which is east of Suriname. In addition, the blue color morph of *D. tinctorius* occurring in the Sipaliwini savanna is potentially vulnerable to rarity-fueled exploitation for the pet trade (Hall et al. 2008).

Despite having been discovered almost 50 years ago (Hoogmoed 1969), the only reported subsequent attempt to ascertain the status of the blue morph of *D. tinctorius* in the Sipaliwini savanna occurred in 1996 (Cover 1997). At that time Cover (1997) found frogs but reported potential evidence of illegal collection. Thus, the primary objective of the current study was to determine the current status (presence or absence) of *D. tinctorius*, in the Sipaliwini savanna. If present, the plan was to establish survey plots and conduct preliminary surveys to serve as benchmarks for future population studies.

Materials and Methods

Survey plots. A survey plot measuring 50 x 50 m was established in each of three forest islands which were

separated by approximately 350–750 m of tall grass savanna. Forest islands were identified using the same numbering scheme as Hoogmoed (pers. comm.) [Fig. 1]. Survey plots within the forest islands measured approximately 4% of 6 ha (Forest Island 1), 12.5% of 2 ha (Forest Island 2), and 6% of 4 ha (Forest Island 4) [Fig. 1]. Forest Island 3, which lies ~300 m north of Forest Island 2, was not surveyed due to time constraints. Plot placements were determined by entering each of the forest islands and visually searching until a *D. tinctorius* was encountered (Fig. 2). The nearest stream was then used as one side of the 50 x 50 m sample plot, based on previous research by Hoogmoed (1969) and Cover (1997) citing stream preference by the frogs. Plot corners were marked with GPS coordinates using a Garmin 60CSx. All three forest islands were subsequently digitized using images from Google Earth Pro, which were further edited in ArcMap, version 10.2, to show the locations where the surveys were conducted. To do this, Google Earth imagery was acquired and added as a layer into ArcMap. Then Arc toolbox's KML layer feature was used to transfer the digitized forest islands created in Google Earth to ArcMap. The resulting image emphasizes the presumed isolation by habitat of the *D. tinctorius* populations from one another (Fig. 1).

Survey plots 1 and 4 were bordered on three sides by small clear water streams. Survey plot 2 was characterized



Fig. 2. *Dendrobates tinctorius* from Sipaliwini savanna, Suriname.

by having a small clear water stream at the lowest part of the plot. Survey plot 1 was located on a north-facing slope that rose from 315–340 m in elevation. The area was under a fully closed canopy forest with dense understory vegetation interspersed with lianas. The forest floor was covered by leaf litter, decomposing fallen trees, and boulders of about 2–3 m in height and 3–6 m in diameter (Fig. 3). Survey plot 2 was on a southeast facing slope that rose from 315–325 m in elevation. The plot was under a closed canopy with an open understory and a forest floor covered by leaf litter. There were no boulders in this plot. Survey plot 4 was on a west-facing slope that rose from 300–350 m in elevation. The area was under a fully closed canopy with dense understory vegetation. One-third of the plot on the southwest side had a dense understory of *Phenacospermum* sp. and *Poaceae* bamboo species. The forest floor was covered by leaf litter interspersed with 10–15 clumped boulders that were about 1–3 m high and 1–5 m in diameter.

Survey methodology. Each plot was visually surveyed once, for varying amounts of time, from 1030–1300 h. Surveys occurred on 16–18 June 2015, three days after the last rainfall on 13 June 2015. To survey a plot, nine individuals were spaced approximately 1.5 m alongside each other forming a line. Starting at one corner of the plot, the surveyors walked 50 m to the opposite side of the plot. Upon reaching the far side the surveyors again spaced laterally starting 1.5 m from the person on the farthest end to cover the next section, and walked back towards the opposite side. This process was repeated until the whole plot was surveyed. Survey participants wore sterile disposable gloves which were changed between the captures of each individual. Capture locations of individual frogs were recorded as GPS coordinates using a Garmin 60CSx. Frogs were then placed in individually labeled Zip-Lock bags. Snout-vent length (SVL) was measured to nearest 0.1 mm with digital calipers. Adults were not sexed. After measurements were taken, each frog was released at its original point of capture.

To identify potential changes in perimeter sizes of the forest islands due to environmental or anthropogenic

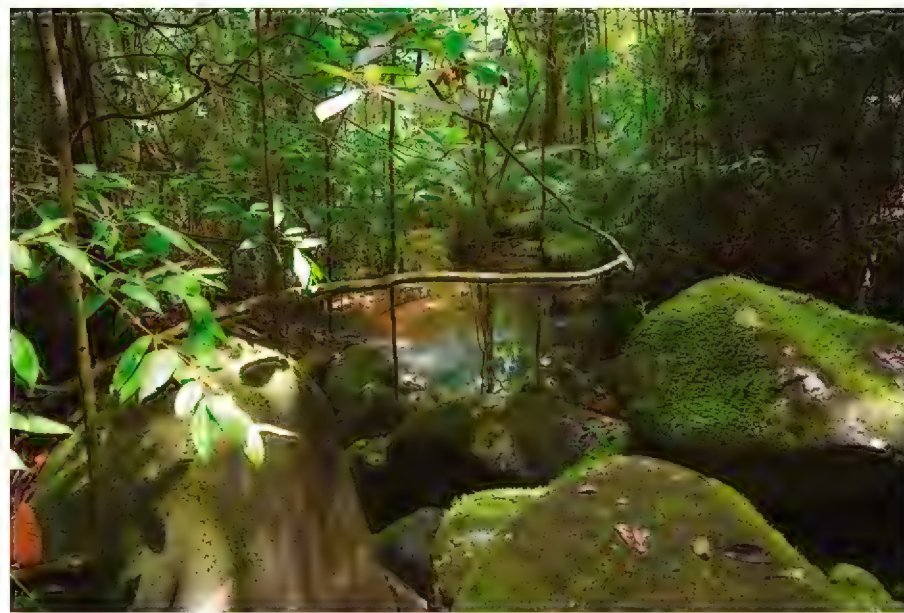


Fig. 3. Stream and boulder habitat in Survey Plot 1, Sipaliwini savanna, Suriname.

factors, the most historical and most recent available Google Earth images from 31 December 1969 and 17 November 2004 were used. These images were visually compared by juxtaposing the forest islands outlined in red, from 2004 (Fig. 4A) onto the 1969 image (Fig. 4B).

Statistical methods. A General linear model ANOVA was used to compare SVL values between specimens from each forest island. A *t*-test was used to compare SVL of frogs measured in the current survey with those reported by Hoogmoed (1969: Table I). SVL are reported to nearest 0.1 mm $\pm$ 1 SD. All analyses were conducted using Minitab 17 statistical software (Minitab Inc., State College, Pennsylvania, USA).

Results

Twenty-two adults and one juvenile were found in the three forest island plots surveyed. Sixteen frogs were found during the surveys, and seven were found adjacent to the survey plots. Two frogs were found in Forest Island 1 in 1 h (270 person-min/frog). Four frogs were found adjacent to the survey plot in Forest Island 1 in 0.5 h (67.5 person-min/frog). Four frogs were found in Forest Island 2 in 0.5 h (67.5 person-min/frog). Ten frogs were found during the survey of Forest Island 4 in 1.5 h (81 person-min/frog). Three frogs were found adjacent to the survey plot in Forest Island 4 in 0.5 h (90 person-min/frog). The overall mean yield of encounters was 115.2 person-min/frog. Variations in time spent conducting a survey often reflected the difficulty in traversing the plot based on the physical obstructions encountered (e.g., boulders and density of herbaceous undergrowth).

Adult SVL values ranged from 36.7–46.4 mm, with a mean of 42.0 ± 3.1 mm. No significant differences between SVL of adult frogs from different forest islands were found (one-way ANOVA: $F_{2,21} = 0.23$, $P = 0.795$). No significant difference was found in a comparison of SVL values recorded by Hoogmoed (1969) and the current survey (*t*-test: $t = 1.49$, $df = 44$, $P = 0.144$). The single juvenile recorded here measured 18.9 mm SVL.

A comparison of the vegetative perimeters of Forest

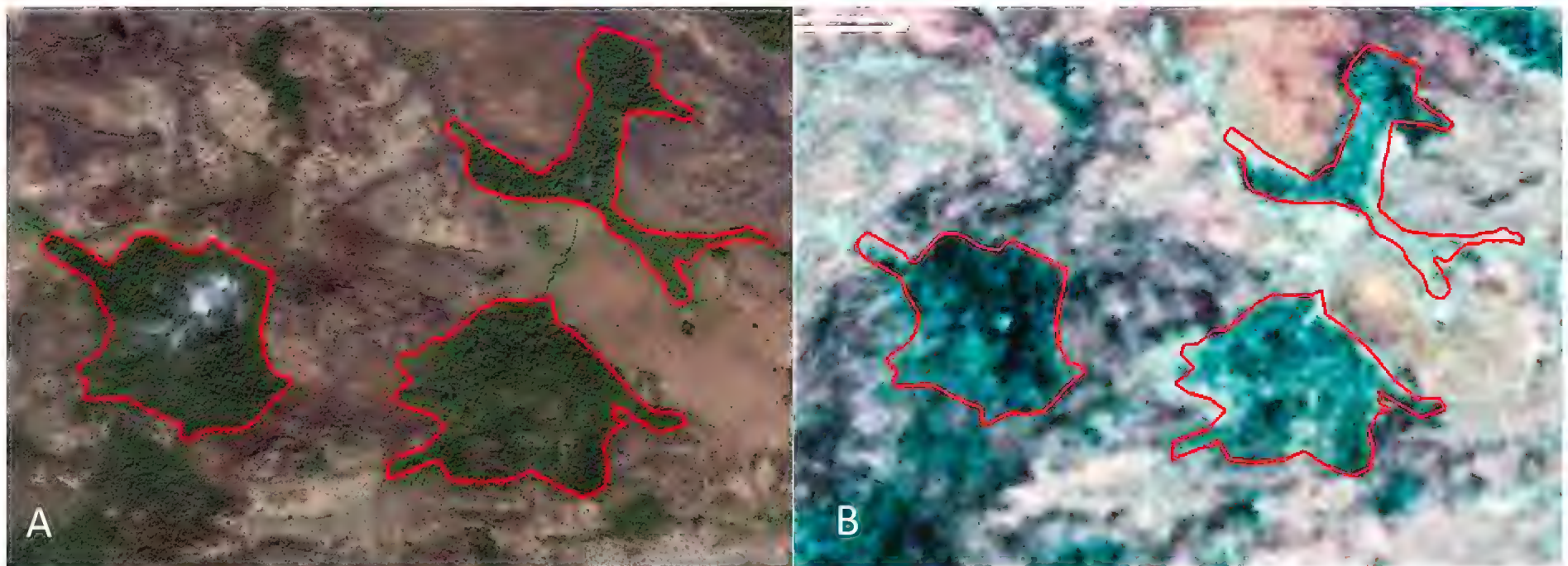


Fig. 4. Forest Islands 1, 2, and 4, extant vegetation comparison between Google Earth images for 2004 (A) and 1969 (B). Red outlines delineating the three forest islands were juxtaposed from the 2004 image onto the 1969 image.

Islands 1, 2, and 4 appear almost identical in the two Google Earth images taken in 2004 (Fig. 4A) and 1969 (Fig. 4B).

Discussion

Survey findings. The survey sites represented three of the four forest islands where frogs were found in the surveys by Hoogmoed (1969) and Cover (1997). In the current surveys only adults were found, with the exception of a single juvenile. No males transporting tadpoles were found. Hoogmoed (1969) reported a male carrying two tadpoles on his back on 30 September 1968, while Cover (1997) who surveyed in June, did not note this behavior, although he reported newly metamorphosed individuals and tadpoles at two sites.

This survey recorded fewer frogs (23) than either Hoogmoed (1969) or Cover (1997). Cover (1997, pers. comm.) reported 56 frogs from Forest Island sites 1, 2, and 4. Hoogmoed (1969, 2019) reported 82 frogs from Forest Islands 1–4, during 11 different dates in 1968 and 1970. Differences in methodologies, season, and weather limit most direct comparisons between these studies. Both Cover (pers. comm.) and Hoogmoed (1969, 2019) concentrated their searches in suitable habitat along stream courses, while in the current survey the 50 x 50 m plots were centered around the spot where a frog was first encountered. Weather events, such as rainfall, presumably influence frog activity. For example, Cover noted that after heavy rains at one site, the numbers of frogs visible on the forest floor increased dramatically (J. Cover, pers. comm.). A similar situation was also reported by Wevers (2007). The current surveys took place 3–5 days after the last rains. Hoogmoed (1969, 2019) reported 82 frogs during herpetological inventories conducted on 11 different days in September and October 1968 and February 1970. In comparing the time required to encounter a frog, Hoogmoed (2019) averaged 10.1 person-min/frog while the average in the current survey was 115.2 person-min/frog. This would suggest a

significant population decline if all other factors were equal.

The blue morph of *D. tinctorius* in the Sipaliwini savanna forest islands appears to be both geographically and genetically isolated from other color morphs of *D. tinctorius* in Suriname. The nearest known populations of *D. tinctorius*, which are black and yellow dorsally, are approximately 23 km north of the closest forest islands surveyed (Fouquet et al. 2015). Blue morphs of *D. tinctorius* in Sipaliwini are more than 300 km away from the blue morphs reported by Silverstone (1975), and approximately 315 km from the blue morphs reported by Avila-Pires et al. (2010). In addition, the frogs in each of the forest islands surveyed may be genetically isolated from each other. Exploration of other remote forest islands in the Sipaliwini savanna region may reveal additional isolated populations of blue *D. tinctorius*. Future molecular analyses could elucidate the genetic divergence in these forest island populations since their presumed isolation approximately 10,000 years ago (Riezebos 1979).

Conservation considerations and future threats. *Batrachochytrium dendrobatidis* (*Bd*) has been recorded in French Guiana (Courtois et al. 2012, 2015) and Brazil (Becker et al. 2016) which border Suriname to the east and south, respectively. During this rapid survey no dead frogs or frogs displaying visual symptoms of *Bd* were found, although seemingly healthy specimens are still known to be carriers of this disease (Coutinho et al. 2015).

While no evidence was found of illegal collecting for the pet trade, that lack of evidence is not proof that it does not occur or will not occur in the future. The indigenous Trio peoples control both access to the transportation to the savanna (canoes) and permission to visit the forest islands where the frogs are found. Trio culture recognizes the uniqueness of these frogs. Permission from the village chief must be granted to visit the sites where frogs may be observed, but collection is not allowed. This is

enforced by the accompanying Trio guides. Presently, the Trio people's stewardship practices provide a large measure of protection from collecting for the pet trade. Sustainable programs that benefit the Trio community should be implemented to reinforce their stewardship of the habitat and wild populations of this unique color morph.

Visual comparison of the perimeter size of these forest islands between the 2004 and 1969 images do not appear to indicate a significant change in size, despite the history of anthropogenic fires (Fig. 4A,B). Cover (1997) reported charred wood on the periphery of the forest islands. However, when he observed a fire in the savanna, it stopped when it reached the lush vegetation on the perimeter of a forest island (J. Cover, pers. comm.). Although the forest islands appear to be stable in size, due to their small area and isolation, any reduction in the water sources either through drought or climate change could threaten both the forest island vegetation and associated stream habitat. An increase in xeric conditions could increase the risk of forest island vegetation becoming drier on the periphery and thus more susceptible to anthropogenic fires resulting in the edges of these islands moving inwards, leading to a decrease in their size or even complete destruction.

Barring direct human collection, climate change may be the most serious threat to this unique blue morph of *D. tinctorius*. There is a growing body of evidence that tropical species will need to undergo elevational or latitudinal range shifts to remain in analogous climatic conditions in the future (Colwell et al. 2008; Nowakowski et al. 2016). Given the isolated nature of these forest islands, their lack of connectivity with either cooler forests or altitudinal refugia, any change to the forest island habitat may be the biggest threat to the continued survival of this unique *D. tinctorius* blue morph in the wild.

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Foundation.

Literature Cited

- Avila-Pires TCS, Hoogmoed MS, Rocha WA. 2010. Notes on the vertebrates of northern Pará, Brazil: a forgotten part of the Guianan Region, I. Herpetofauna. *Boletim do Museu Paraense Emílio Goeldi. Ciências Naturais* 5: 13–112.
- Becker CG, Rodriguez D, Lambertini C, Toledo LF, Haddad CF. 2016. Historical dynamics of *Batrachochytrium dendrobatidis* in Amazonia. *Ecography* 39: 954–960.
- Colwell RK, Brehm G, Cardelús CL, Gilman AC, Longino JT. 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* 322: 258–261.
- Coutinho SDA, Burke JC, Paula CDD, Rodrigues MT, Catão-Dias JL. 2015. The use of singleplex and nested PCR to detect *Batrachochytrium dendrobatidis* in free-living frogs. *Brazilian Journal of Microbiology* 46: 551–555.
- Courtois EA, Pineau K, Villette B, Schmeller DS, Gaucher P. 2012. Population estimates of *Dendrobates tinctorius* (Anura: Dendrobatidae) at three sites in French Guiana and first record of chytrid infection. *Phyllomedusa* 11: 63–70.
- Courtois EA, Gaucher P, Chave J, Schmeller DS. 2015. Widespread occurrence of Bd in French Guiana, South America. *PLoS ONE* 10: e0125128.
- Cover JF. 1997. A pilot field study of the blue poison-dart frog *Dendrobates azureus* in Suriname. *American Zoo and Aquarium Association, Regional Conference Proceedings* 1997: 492–498.
- Fouquet A, Jean-Pierre V, Kadosoe V, Ouboter P, Jairam R. 2015. Checklist of the amphibians of the Sipaliwini area, Suriname. *Herpetology Notes* 8: 63–68.
- Frost DR. 2017. Amphibian species of the world: an Online Reference. Version 6.0. Available: <http://research.amnh.org/herpetology/amphibia/index.html> [Accessed: 16 November 2017].
- Hall RJ, Milner-Gulland EJ, Courchamp F. 2008. Endangering the endangered: the effects of perceived rarity on species exploitation. *Conservation Letters* 1: 75–81.
- Hoogmoed MS. 1969. Notes on the herpetofauna of Surinam III. A new species of *Dendrobates* (Amphibia, Salientia, Dendrobatidae) from Surinam. *Zoologische Mededelingen* 44: 133–141.
- Hoogmoed MS. 1973. *Notes on the Herpetofauna of Surinam IV. The Lizards and Amphisbaenians of Surinam*. Biogeographia 4. Brill, Leiden, Netherlands. 419 p.
- Hoogmoed MS. 2019. Unpublished population data of *Dendrobates azureus* Hoogmoed 1969 obtained in 1968 and 1970, and its historical and current taxonomic status. *Amphibian & Reptile Conservation* 13(2

[General Section]): 95–109 (e190).

James TY, Toledo LF, Rödder D, Leite DS, Belasen AM, Betancourt-Román CM, Jenkinson TS, Soto-Azat C, Lambertini C, Longo AV, et al. 2015. Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: lessons from the first 15 years of amphibian chytridiomycosis research. *Ecology and Evolution* 5: 4,079–4,097.

Lynden AJH van. 1939. Op zoek naar de Zuidgrens. De grensbepaling tusschen Suriname en Brazilië, 1935–1938. *Tijdschrift van het Koninklijk Nederlandsch Aardrijkskundig Genootschap* 56: 1–90.

Noonan BP, Gaucher P. 2006. Refugial isolation and secondary contact in the dyeing poison frog *Dendrobates tinctorius*. *Molecular Ecology* 15: 4,425–4,435.

Nowakowski JA, Watling JI, Whitfield SM, Todd BD, Kurz DJ, Donnelly MA. 2016. Tropical amphibians in shifting thermal landscapes under land-use and climate change. *Conservation Biology* 31: 96–105.

Oldenburger FHF, Norde R, Riezebos HTh. 1973. *Ecological Investigations on the Vegetation of the*

Sipaliwini Savanna Area (Southern Suriname). Institute of Systematic Botany Utrecht, Netherlands. Available: http://www.sipaliwinisavanna.com/docs/ecological_investigations_vegetation_sipaliwini_savanna.pdf [Accessed: 16 November 2017].

Ouboter PE, Jairam R. 2012. *Dendrobates tinctorius azureus*. Pp. 106–107 In: *Amphibians of Suriname*. Brill, Leiden, Netherlands. 376 p.

Riezebos HT. 1979. *Geomorphology and Soils of Sipaliwini Savanna, Southern Suriname*. Geografisch Instituut, Rijksuniversiteit Utrecht, Utrecht, Netherlands. 168 p.

Silverstone PA. 1975. A revision of the poison-arrow frogs of the genus *Dendrobates* Wagler. *Natural History Museum of Los Angeles County. Science Bulletin* 21: 1–55.

Wevers E. 2007. In het biotoop van de okopipi. *Onder het Palmblad* 10: 13–23.

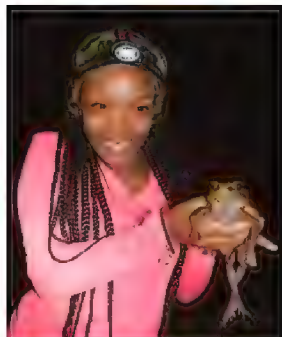
Wollenberg KC, Veith M, Noonan BP, Lötters S. 2006. Polymorphism versus species richness – systematics of large *Dendrobates* from the Eastern Guiana shield (Amphibia: Dendrobatidae). *Copeia* 2006: 623–629.



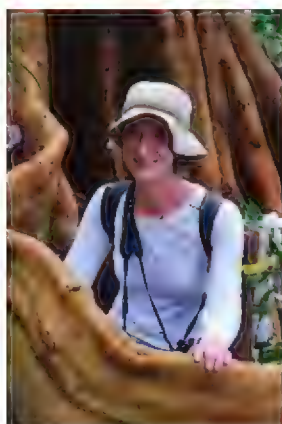
Christian d'Orgeix is a behavioral ecologist and conservation biologist at Virginia State University. Christian's research focuses on the mating systems and conservation biology of reptiles and amphibians. One of his current projects examines the probability of extinction of high- and low-elevation populations of lizards. In Suriname, Christian and his students collaborate with Dr. Paul Ouboter and Rawien Jairam from Anton de Kom Universiteit, Suriname, on studies on the behavior and conservation of frogs and lizards.



Rawien Jairam works at the National Zoological Collection of Suriname and is co-author of the book *Amphibians of Suriname*. Rawien has an M.Sc. in Conservation Biology and has been interested in the herpetofauna of Suriname for many years. Apart from general herpetology, he is specifically interested in taxonomy and species distributions.



De'Jah T. Hardy is a junior Biology major at Virginia State University. De'Jah traveled to Suriname, South America, to conduct research on frogs and ants. She also traveled to China to take Clinical Medicine classes at Jinan University in Guangzhou. De'Jah's goal is to explore as many opportunities as she can as an undergraduate, and then go straight for her Master's in Psychology. She plans to travel the world someday, helping children as a traveling Occupational Therapist.



Sarah Melissa Witiak is a plant biologist who received her Ph.D. from Pennsylvania State University (University Park, Pennsylvania, USA) and currently teaches at Virginia State University. Sarah is primarily interested in the “pretty” parts of biology, including poison-arrow frogs, insect galls, and flower evolution and ecology. Her current projects include studies of plant volatiles and a survey of insect gall diversity in Suriname.

Laren Robinson worked as a GIS specialist at Virginia State University. She is currently pursuing career options in GIS applications. (No photo available)



Book Review

Night Lizards: Field Memoirs and a Summary of the Xantusiidae

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Keywords. Behavior, biogeography, ecology, reproduction, reptiles, Scincomorpha, Squamata

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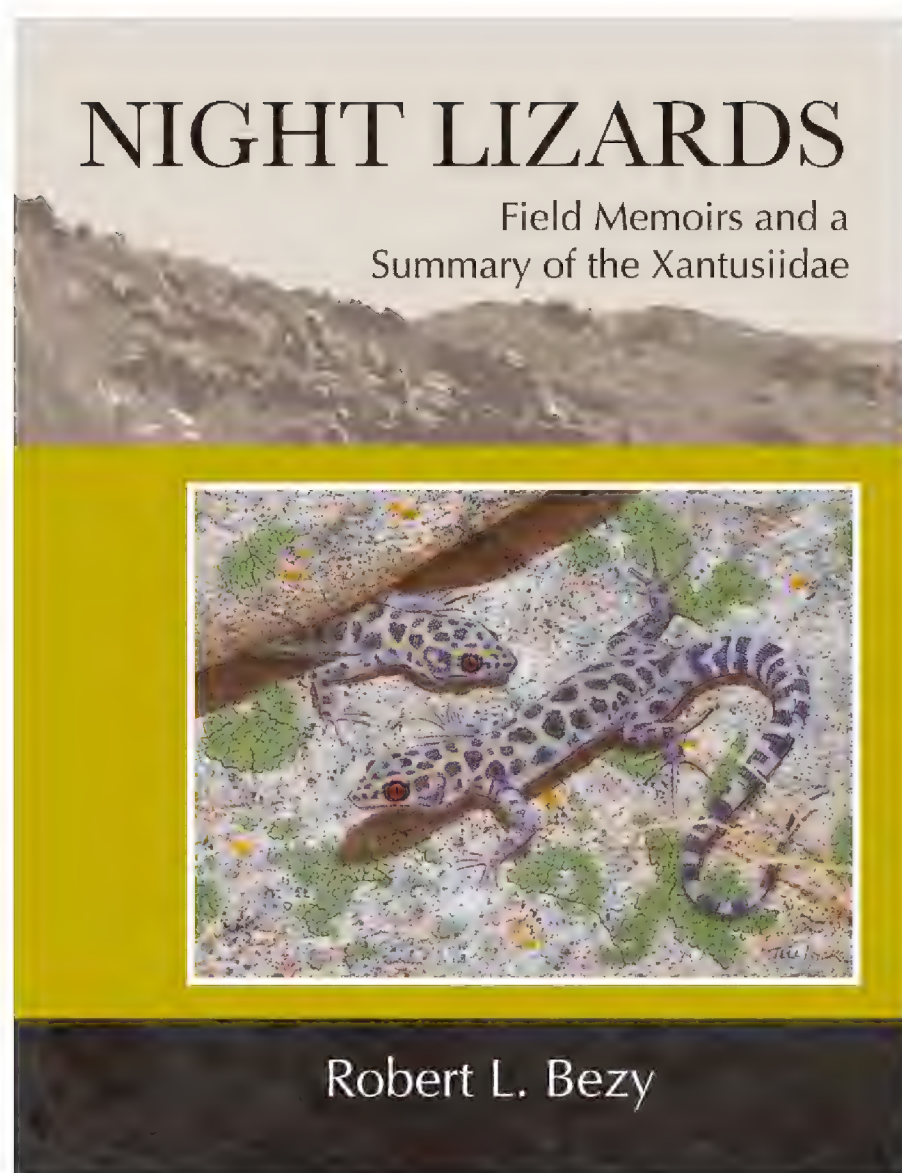
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Herpetologist Robert L. Bezy has produced a fascinating memoir of his life and the lives of night lizards. When reading about or researching night lizards, Bezy's name comes up often. When Bezy began his career there were only a few night lizards known to science; now there are 35 living species in the family Xantusiidae. The book begins with a brief autobiography, detailing what motivated Bezy to pursue herpetology and what became a long and winding road full of exciting discoveries and working with some of the best herpetologists in the field. Some of my favorite sections are where Bezy divides his narrative into “locational highlights” and tells stories of some experiences that had significant impacts on his life. These stories are rich in landscape descriptions, wildlife encounters, and how the adventure—planned or not—influenced the way he views life. These sorts of reflections are priceless and allow the reader to reflect on his or her own life. As I read through these stories I found myself frequently reminiscing about my various encounters with night lizards and other wildlife.

Following the autobiography and locational highlights, Bezy delves into the historical perspectives regarding night lizards. A “must read” is the account of János Xántus and Spencer Fullerton Baird. Baird named the night lizard genus (*Xantusia*) and family (*Xantusiidae*) after Xántus and interesting enough Xántus later wrote to Baird that he didn't even remember collecting the small lizard. Other important perspectives include those of John Van Denburgh, Edward H. Taylor, Hobart M. Smith, Jay M. Savage, Robert G. Webb, Richard G. Zweifel, and Charles H. Lowe. All of these men contributed to the natural history of the night lizards and Bezy does a splendid job recapping their contributions.

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Title: *Night Lizards: Field Memoirs and a Summary of the Xantusiidae*

Authors: Tell Hicks (Artist), Robert Bezy (Author)

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Pages: ii + 220; **Price:** USD \$24.95



Fig. 1. *Xantusia vigilis* from the Mojave Desert, Los Angeles County, California. Photo by Howard O. Clark, Jr.

Another highlight of the book is the “questions” section. Here, Bezy addresses a variety of natural history aspects of the night lizard—undoubtedly questions that he had over the years and now the reader has the opportunity to read the answers with Bezy as the messenger. Topics include rock crevice ecology of the *Xantusia* and *Lepidophyma*; night lizards in caves; island gigantism; the species concept; the idea of unisexuals; night lizard ecology; are night lizards nocturnal?; reproduction; sociobiology; diet and predators; helminth parasites; thermal and water ecology; movement, home range, and population density; and conservation status.

The last half of the book provides a detailed discussion about the night lizard family, Xantusiidae, followed by the night lizard species accounts. Originally, the family only had one species, *Xantusia vigilis* (Fig. 1), the small lizard János Xántus collected at Fort Tejon, California. But, eventually two other genera were added, *Cricosaura* (Cuban night lizards), and *Lepidophyma* (tropical night lizards). Bezy provides a detailed map that shows the distribution of the three genera and a diagram showing the phylogenetic relationships. From there, each species has its own detailed account, which generally includes these sections: identification; chromosomes; size; distribution and habitat; life history; sex ratio; etymology; conservation status; and discussion. Each account has a color photo of the lizard, a colored distribution map, and photos of representative habitat. With nearly 250 total figures and photos throughout the book, the reader is treated to a photo library that is unbeatable. Following the species accounts is a night lizard species key—complete with diagrams and photos. Also included are scale features for differentiating night lizard species. The book ends with a literature cited section which likely includes all the key papers ever written on night lizards.

Overall, Bezy’s book is a must read for anyone interested in the story behind the night lizard, or in tales of herpetological discovery and adventure in general. The storytelling alone is reason enough to buy the book; the photos, species accounts, range maps, etc., are a super bonus and make the book the best resource currently on this topic.



Howard O. Clark, Jr. has more than 20 years of professional wildlife and research experience. Howard is certified by the Ecological Society of America as an ecologist and by The Wildlife Society (TWS) as a Certified Wildlife Biologist®. His work as an ecological consultant has focused on the fauna and ecosystems of California and has included extensive baseline inventories, surveys for rare animals, and habitat assessments. He has conducted dozens of inventories, surveys, and assessments for Blunt-nosed Leopard Lizard, Western Burrowing Owl, San Joaquin Kit Fox, Giant Kangaroo Rat, and Mohave Ground Squirrel among many others. Howard developed his consulting skills while working for H. T. Harvey & Associates (Los Gatos, California) for 10 years and Garcia and Associates (Auburn, California) for three years. He currently works for Colibri Ecological Consulting, LLC, as a Senior Scientist in Fresno, California. Prior to working as a consultant, Howard spent seven years as a wildlife biologist with the Endangered Species Recovery Program (California State University, Stanislaus Foundation, Turlock, California). He completed his Master’s degree at CSU, Fresno in 2001. His thesis addressed the interactions between the endangered San Joaquin Kit Fox and the non-native Red Fox in Kern County, California. Howard is an instructor for TWS kit fox and small mammal workshops, and the Western Section of TWS awarded him the Raymond F. Dasmann Award for Professional of the Year in 2015. He is the Layout Editor for the Western Section’s journal, *Western Wildlife*, as well as three herpetological journals: *Amphibian & Reptile Conservation*, *Sonoran Herpetologist*, and *Herpetological Conservation and Biology*. During leisure time, Howard enjoys hiking, geocaching, and visiting places of historical interest with his daughter.



Impacts of a highway on the population genetic structure of a threatened freshwater turtle (*Glyptemys insculpta*)

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Abstract.—Genetic partitions for members of the family Emydidae often correspond with both natural and anthropogenic landforms. For semi-terrestrial turtles, clear negative impacts are associated with habitat fragmentation via roadways, such as loss of breeding individuals, increased inbreeding, and decreased migration. The Wood Turtle (*Glyptemys insculpta*) is a Species of Special Concern in New York and native to the central portion of the state, where Interstate Highway 88 was constructed in the 1970s. To examine possible impacts of the highway on local populations, a museum collection of Wood Turtles that predates road construction was used. Specifically, microsatellite markers were used to compare historic ($n = 38$) and contemporary ($n = 26$) Wood Turtle DNA from opposite sides of the highway. The measured parameters were inbreeding (F_{is}), differentiation (F_{st}), number of breeding individuals (N_b), migration (m), and overall population genetic structure. The populations on either side of the highway were predicted to have become more differentiated and inbred over time, and migration was predicted to decrease over time. Overall, populations on either side of the interstate were historically a single population, had a greater number of breeding individuals, and were less differentiated. No change in inbreeding was found across time. These findings suggest there is more migration, running north to south between the two populations, likely attributable to the directionality of the flow associated with local creeks. Further research examining these two separate populations within the context of the entire state is necessary to determine whether they should be treated as separate Conservation Units.

Keywords. Emydidae, habitat fragmentation, roadways, microsatellite, population structure

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Introduction

On a global scale, amphibians and reptiles are in decline due to pressures which include climate change, unsustainable harvest, habitat loss, and habitat degradation (Gibbons et al. 2000). Among all reptile and amphibian species, members of the order Testudines are particularly vulnerable to increased decline when faced with increasing anthropogenic disturbances, such as road mortality and illegal harvesting (Lieberman 1994; Garber and Burger 1995; Wood and Herlands 1997; Williams 1999; Levell 2000; Gibbons et al. 2000; Gibbs and Shriver 2002; Steen and Gibbs 2004; Gibbs and Steen 2005; Steen et al. 2006; USFWS 2015). Nearly half of all turtle species are currently categorized as threatened or endangered (Rhodin et al. 2011). Terrestrial turtles are generally perceived as poor long-distance dispersers. Limitations to dispersal enable habitat fragmentation, which can put populations at risk of extinction due to demographic and genetic diversity loss (Gibbons et

al. 2000). In North America, several case studies have suggested that anthropogenic disturbances, particularly roadways, have direct negative impacts on freshwater turtles by skewing sex ratios and increasing the mortality of migrating individuals (Buhlman and Gibbons 1997; Wood and Herlands 1997; Williams 1999; Levell 2000; Gibbons et al. 2000; Gibbs and Shriver 2002; Steen and Gibbs 2004; Gibbs and Steen 2005; Steen et al. 2006; USFWS 2015).

When assessing the negative impacts of fragmented populations, genetic markers can identify dispersal pathways and population diversity (Lamb et al. 1989; Galbraith et al. 1995). For example, studies focusing on turtle populations have found river drainages and intermontane basins to be barriers to gene flow (Gibbs and Amato 2000). Similarly, several studies have identified relatively high allelic diversity in Wood Turtle populations when compared to other species (Gibbs 1993; Tessier et al. 2005; Amato et al. 2008; Castellano et al. 2009; Spradling et al. 2010). Given

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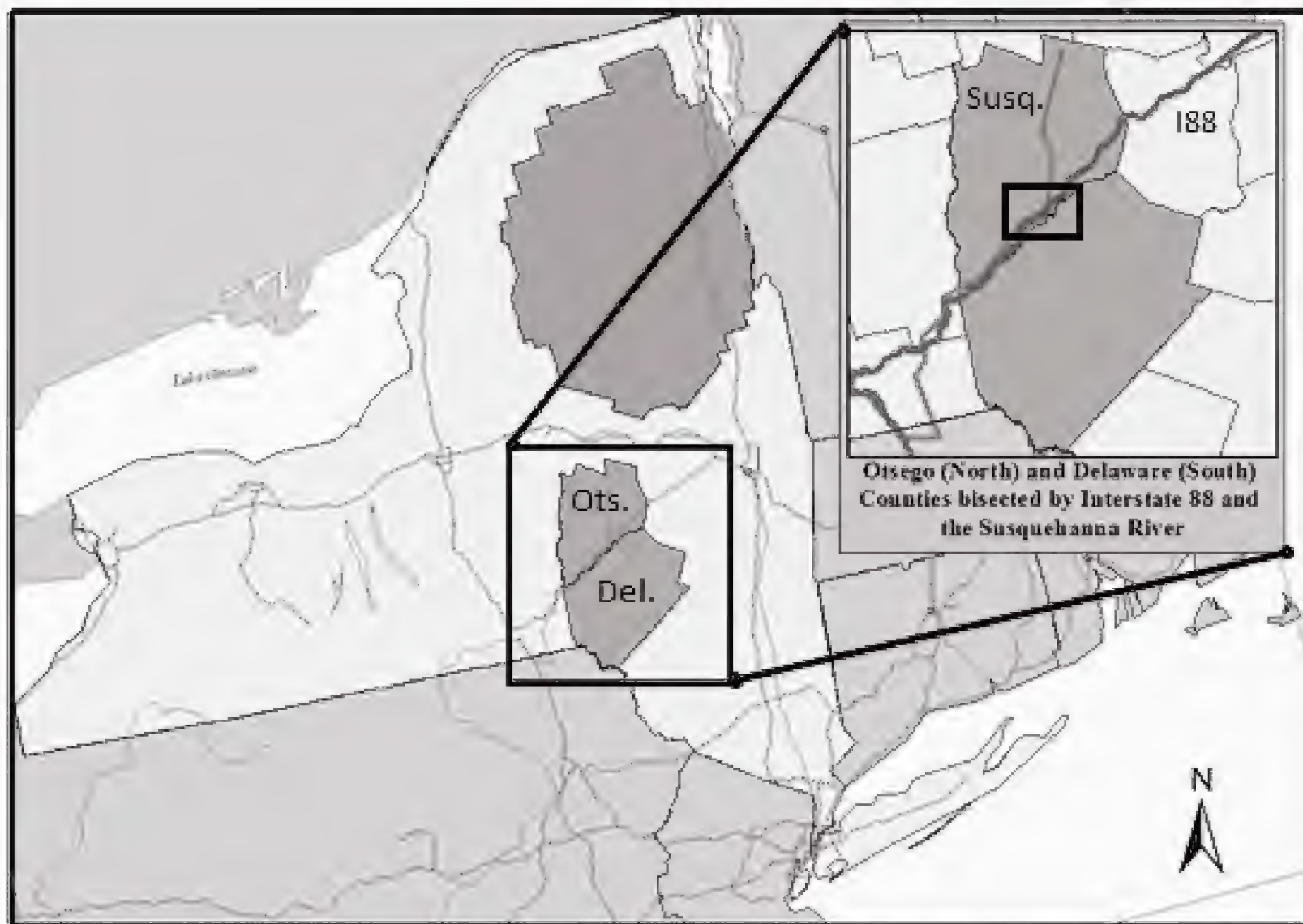


Fig. 1. Study area. Interstate Highway 88 (I-88) and the Susquehanna River (Susq.) bisect Otsego and Delaware Counties, New York, USA.

their poor long-distance dispersal ability, populations of freshwater turtles could be at increased risk from habitat fragmentation via natural or anthropogenic barriers, which may result in loss of genetic and demographic connectivity (Gibbons et al. 2000; Gibbs and Amato 2000). Specifically, turtles which do attempt to move long distances within a fragmented landscape, as females often do for nesting, may be at greater risk of dispersal related mortality, e.g., roadkill (Gibbs and Shriver 2002; Steen and Gibbs 2004). Road mortality driven by habitat fragmentation is believed to be the culprit for the gradual skewing of sex ratios and the general decline of female turtles among many freshwater turtle species throughout the United States (Steen and Gibbs 2004; Gibbs and Steen 2005; Steen et al. 2006).

Known to disperse both long and short distances (Harding and Bloomer 1979) throughout its range, the Wood Turtle (*Glyptemys insculpta*) is a Species of Special Concern in New York State, which may be at risk from the demographic and genetic impediments associated with habitat fragmentation (Gibbons et al. 2000; Breisch and Behler 2002; Tuttle and Carroll 2003; Arvisais et al. 2004; Sweeten 2008). Limitations to dispersal within fragmented landscapes are believed to contribute to the decline of wetland-dependent turtles (Gibbs 1993; Tessier et al. 2005; Amato et al. 2008; Castellano et al. 2009; Spradling et al. 2010). Although previous studies have examined Wood Turtle population genetics, none have focused on the potential impacts that anthropogenic

habitat fragmentation could have on regional populations (Tessier et al. 2005; Amato et al. 2008; Castellano et al. 2009; Spradling et al. 2010).

Between 1974 and 1980, Interstate Highway 88 (I-88, 193 km) was built across eight Central New York counties (Fig. 1), including Otsego and Delaware counties (Associated Press 1986; Edwardsen 1989). Prior to this, from 1958–1968, Dr. John New collected and dry-preserved Wood Turtles ($n = 300$) from across New York state, including sites north and south of I-88. Using this historic data set in conjunction with contemporary data, this study examines the potential impacts of building a large interstate highway on a vulnerable turtle species over a 60-year period. Over that same 60-year period, New York State has become more populated, according to U.S. Bureau of Census data for 1960–2010. Given that wetland habitat size and connectivity degrade with increased human activity (Gibbs 2000), and that such disruption of wetland mosaics can have dramatic negative impacts on semi-terrestrial turtle populations (Gibbs and Shriver 2002), declines are expected to be observable on the genetic scale. Specifically, an increase in genetic differentiation between populations (F_{st}), a decrease in the effective breeding population size (N_e) between sampling sites on either side of Interstate 88, and limited gene flow between populations on either side of the highway are expected. Here, microsatellite data are used to examine these critical genetic parameters.

Materials and Methods

Study sites: Contemporary ($n = 26$) and historic ($n = 38$) data were collected from two streams in Otsego County and two streams in Delaware County, New York. The furthest sections of the two Otsego County streams sampled are rectilinearly 11 km and 28 km north of Interstate 88. The furthest sections of the two Delaware county streams sampled are rectilinearly 3 km and 9 km south of Interstate 88. All streams sampled are part of the Susquehanna watershed, and terminate on the southern side of Interstate 88 (Fig. 1). Contemporary sites were sampled during the spring/early summer and late fall active periods of 2015 and 2016 using the Regional Conservation Needs protocol, which involves sampling in 1 mi increments (Jones et al. 2015).

Samples: Blood samples were used for contemporary data, and 1 mm tail tips were harvested from dried historic specimens. Blood samples (0.1–0.5 ml) were collected from the dorsal coccygeal vein using a sterile 1.0 ml 25-gauge syringe (Jones et al. 2015). Blood was transferred into test tubes immediately upon return from the field and stored in 1:1 1 x PBS buffer in a -20 °C freezer. Genomic DNA was extracted from each sample using the QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, California, USA). Tail tips were digested in Proteinase K for 36 h. Each extracted sample was stored in a -20 °C freezer. Seven microsatellite loci were examined (GmuD16 [Genbank accession number: AF516235], GmuD40 [AF517244], GmuD51 [AF517239], GmuD87 [AF517244], GmuD88 [AF517245], GmuD93 [AF517248], and GmuD95 [AF517249]) using primers initially designed for a close relative of the Wood Turtle, *Glyptemys muhlenbergii* (King and Julian 2004). Samples were amplified using the QIAGEN Multiplex PCR kit (Qiagen, Inc., Valencia, California, USA) and a modified version of the PCR protocol (Castellano et al. 2009). The length of the extension step from this protocol was doubled to optimize historic sample amplification due to the highly-fragmented nature of this DNA. PCR products were analyzed at the Cornell Biotech Institute in Ithaca, New York, and visualized using GENEMARKER version 2.6.7 (Hulce et al. 2011).

Statistical analysis: MICROCHECKER version 2.2.3 was used to test each locus for the presence of null alleles, scoring errors, and large allele dropout (Van Oosterhout et al. 2004). Clustering was used to assign individuals to populations using the program STRUCTURE version 2.2 (Pritchard et al. 2000; Falush et al. 2003). Data were analyzed for all contemporary and historic turtles from each of the sites north (Otsego County) and south (Delaware County) of Interstate 88. For each analysis, three runs were used for each value of K (number of assumed populations) ranging between one and nine.

A 106 burn-in period was used, and 106 Markov Chain Monte Carlo (MCMC) iterations were used in the default “admixture model” of ancestry and correlated allele frequencies. Population origin data (north and south) were provided for each individual. Mean log likelihood and DK values were used to assign individuals to populations [K] (Evanno et al. 2005).

Deviations from the Hardy-Weinberg (HWE) expectation among pairs of loci were tested along with mean heterozygosity, allelic richness, numbers of private alleles, inbreeding coefficient (F_{is}), genetic differentiation (F_{st}), and effective population size (N_e) using GenAlEx version 6.5 (Peakall and Smouse 2006, 2012) for the populations north (Otsego) and south (Delaware) of Interstate 88. The comparison of historic and contemporary loci F_{is} was made using a student's t -test. Evidence of a bottleneck on the contemporary data was tested using the program BOTTLENECK (version 1.2.02, Cornuet and Luikart 1996) with an infinite allele model (IAM) and the two-phase model (TPM) recommended by Luikart et al. (1998) over 10,000 iterations. The significance of Wilcoxon test score output ($\alpha = 0.05$) and mode shift were both used as evidence of a bottleneck (Cornuet and Luikart 1996; Luikart et al. 1998; Chiucchi and Gibbs 2010). Short-term migration (m) between the “last few generations” was estimated using Bayesian inference software BAYESASS (version 3.03, Wilson and Rannala 2003) using 3×10^7 iterations with two long-chains sampling every 2,000 iterations, and this included a burn-in of 107. Specifically, a time span reaching back < 5 generations, or 25–125 years (Chiucchi and Gibbs 2010) was used since the estimated generation time for Wood Turtles is 25 years (Farrell and Graham 1991; Galois and Bonin 1999). Multiple independent model runs were made using a random seed, with final selection made based on Maximum Likelihood (e.g., Chuicchi and Gibbs 2010).

Results

Six of the seven microsatellites amplified consistently. The exception was GmuD51, which was removed from further analyses. Historic specimens had a high allele dropout rate (46%), which is expected for highly fragmented antique DNA (Mills et al. 2000; Sefc et al. 2003). No evidence of genotyping error or null alleles was found for those that amplified. Sample size, effective population size, heterozygosity, and overall differentiation between north and south populations, for both historic and contemporary data, are summarized in Table 1. F_{st} values for the contemporary populations was 0.166, while historically they were estimated at 0.081 (Table 2). Heterozygosity estimates per locus are summarized in Table 2. Northern contemporary and historic data each showed three of six loci out of HWE. Southern contemporary data displayed four of six loci

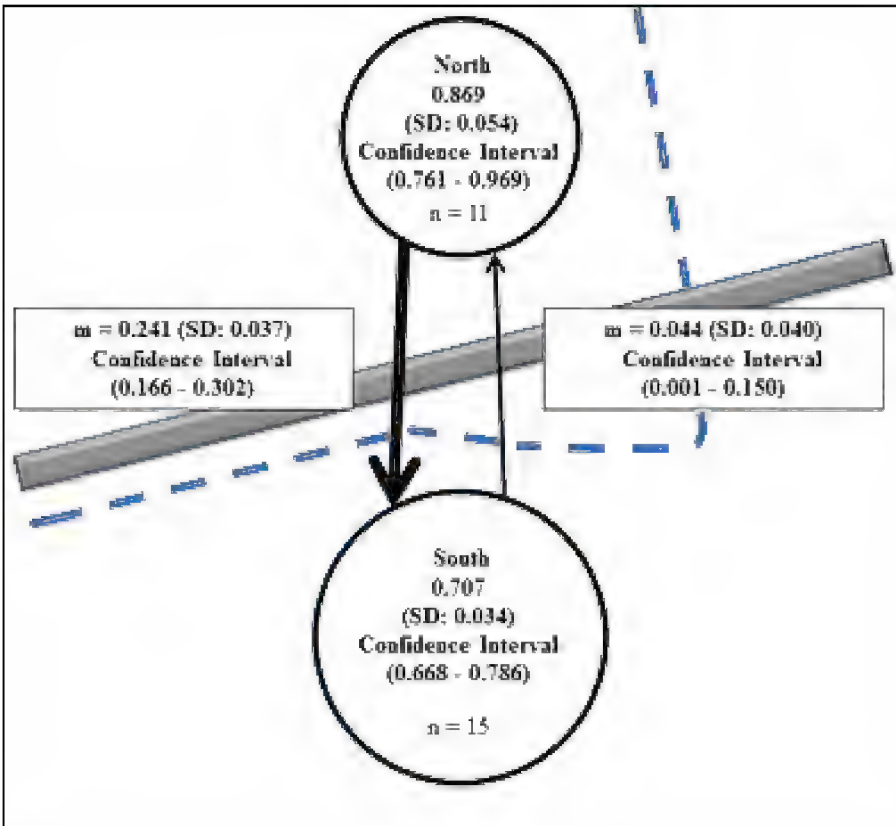


Fig. 2. Estimate of short-term gene flow among populations north and south of Interstate Highway 88 (gray bar) and the Susquehanna River (dashed line) shown with 95% confidence intervals. Circle size reflects relative sample size. Values inside of circles represent the contribution of gene flow from within populations.

out of HWE, while the southern historic data displayed a single locus out of HWE. Comparison of historic and contemporary inbreeding (F_{is}) indicated no difference between estimations ($P = 0.30$). Fixation indexes for each locus are summarized in Table 3.

Contemporary samples clustered into two populations ($K = 2$, $\text{Ln P(D)} = -659.5$, $\text{Var} [\text{LnP(D)}] = 74.5$), with a clear distinction between north and south samples. Historic samples consistently clustered into a single population ($K = 1$, $\text{LnP(D)} = -787.9$, $\text{Var} [\text{LnP(D)}] = 33.0$). There was a deficiency of heterozygosity ($P = 0.04$) for the northern contemporary population under the Wilcoxon rank sign test in the TPM model. Specifically, none of the northern loci were in mutation-drift equilibrium, as five of six loci showed signs of heterozygosity deficiency with the final locus in excess under the IAM model. Southern contemporary populations showed no sign of a genetic bottleneck. Short-term migration (m) was conservatively estimated

to be higher going from north to south (24%) than south to north (4.4%) [Fig. 2].

Discussion

Genetic changes and trends in turtle populations may be difficult to detect due to their naturally long generation times and long lives (Gibbs and Amato 2000). Using the genetic material available from historic samples allowed the successful detection of changes between the populations across a relatively brief period of time. Although this study used a limited number of microsatellites (six of seven), the polymorphic nature of the markers and highly differentiated level of the populations suggest that the results capture an adequate amount of information for comparative genetic analysis (Kalinowski 2002, 2005; Arthofer et al. 2018), especially given the sample sizes for the historic and contemporary populations (Hale et al. 2012). Specifically, the results indicated that these local populations have likely become genetically fragmented over the last 60 years. This may indicate that certain freshwater turtle populations are more vulnerable, in terms of the rate of change, to shifts in genetic structure than previously thought.

Structural analysis of the contemporary data revealed that Wood Turtle populations clustered into two populations, where historically, they were likely a single interconnected unit. In addition, the same samples revealed that local populations have become more differentiated over time as an increase of F_{st} was observed from 0.081 in historical to 0.166 in contemporary populations. This shift from moderate differentiation (> 0.05) to great differentiation (> 0.1) over an evolutionarily short period of time would appear to be aberrant when compared to previous studies examining Wood Turtle differentiation (Hartl and Clark 1997). However, Tessier et al. (2005) sampled Wood Turtles in a similar semi-montane habitat, and found that some of their populations, which had a proximity between sites comparable to those in the current study (~15–50 km), had similar differentiation as the historic samples studied here. Conversely, the contemporary sample differentiation found here is more similar to that of their sites which were much further apart

Table 1. Summarized outputs of population parameters from GenAlEx v 6.5. Parameters displayed are sample size (n), effective population size (N_e), observed (H_o) and expected (H_e) heterozygosity, and overall differentiation (F_{st}) between North and South collecting sites.

Historic (1955-1965)							
	<i>n</i>	N _e (SE)	Mean # alleles (SE)	H _o (SE)	H _e (SE)	Private alleles	F _{st}
North	20	6.9 (1.5)	10.0 (1.5)	0.61 (0.11)	0.82 (0.04)	40	0.081
South	18	5.3 (1.1)	7.8 (1.6)	0.65 (0.14)	0.77 (0.04)	27	
Total	38	12.2					
Contemporary (2015-2016)							
North	11	4.0 (0.4)	6.3 (0.5)	0.67 (0.04)	0.74 (0.02)	12	0.166
South	15	7.0 (0.8)	11.0 (0.8)	0.81 (0.04)	0.85 (0.02)	19	
Total	26						

Table 2. Summarized contemporary observed (H_o) and expected (H_e) heterozygosity.

Locus	Size range (bp)	# of alleles	North		South	
			H_o	H_e	H_o	H_e
GmuD16	165–296	24	0.700	0.830	0.857	0.829
GmuD87	238–394	24	0.700	0.840	0.857	0.768
GmuD88	114–264	30	0.733	0.849	0.867	0.838
GmuD93	125–389	20	0.333	0.736	0.714	0.885
GmuD95	122–266	24	0.778	0.833	0.667	0.871
GmuD40	157–280	21	0.778	0.747	0.917	0.892

(> 60 km) than the sites in this analysis (Tessier et al. 2005). Overall, this suggests the populations examined here, which are relatively close to one another in terms of physical distance, possess genetic differentiation that is more akin to areas further apart, implying that there is a barrier preventing genetic exchange between them. In flatter areas along the coastal plains of the northeastern United States, differentiation among Wood Turtle populations is essentially non-existent, enabling populations to be panmictic across separation distances greater than 40 km (Castellano et al. 2009). This leads us to believe that dispersal limitation is due to some environmental factor, and not simply life history.

A study on another terrestrial emydid turtle, *Terrapene ornata* (Ornate Box Turtle), in Texas found that a major highway built in 1937 was likely the cause of significant differentiation between populations on either side, but not the cause of a change in overall structure (Richtsmeier et al. 2008; Cureton et al. 2014). Similarly, Tessier et al. (2005) found that the St. Lawrence River acts as a barrier between Wood Turtles on either side of its shores, separating them structurally. It is possible that the observed structural separation between the populations studied here has been compounded by the combination of the intertwining bisection of Interstate 88, and the Susquehanna River (Fig. 1).

Despite the findings of Tessier et al. (2005), the migration analysis output in this study (Fig. 2) may suggest that flooding events are allowing at least some unidirectional gene flow to persist between the populations on either side of Interstate 88. In short, local hydrology from lower order streams at both the north

and south sampling sites terminate at the Susquehanna River south of Interstate 88. Research by Jones and Sievert (2009) indicates that flooding events, which have dramatic impacts on Wood Turtles, may play a vital role in connectivity. Specifically, flood events can displace a substantial (40%) portion of Wood Turtle subpopulations by long distances (1.4–16.8 km) downstream (Jones and Sievert 2009), which may explain the unidirectionality of the migration estimates found here (Fig. 2).

This possibility seems even more likely when considering the local footprint of Interstate 88, much of which is built on steep and sometimes craggy mounds protruding from the stream and forest surface. These mounds, which span four total lanes and occasionally split with a center depression or open fall at the median strip, most likely make terrestrial genetic exchange near impossible between the north and south populations. Moreover, the Susquehanna in its entirety is substantially narrower than the St. Lawrence River (Kammerer 2005), which may make survival of flooding events more likely. Additionally, we observed Wood Turtles using the Susquehanna's embankments and flood plains with some regularity, so although its flow may prevent and influence movement, it should not be considered an insurmountable genetic barrier like the much larger St. Lawrence (Tessier et al. 2005). As such, it is likely that Wood Turtle movement is influenced by the directionality of the flow in creeks and rivers, and may explain the unidirectional migration observed here (Fig. 2).

Although some streams and creeks in Delaware County run north to south as they percolate down from the Catskill mountains, the northern Delaware drainages

Table 3. Microsatellite fixation indexes for both historic and contemporary populations.

Locus	Historic			Contemporary		
	F_{is}	F_{it}	F_{st}	F_{is}	F_{it}	F_{st}
GmU16	0.245	0.308	0.084	0.093	0.226	0.146
GmU87	-0.246	-0.045	0.161	-0.056	0.102	0.150
GmU88	0.253	0.282	0.038	0.030	0.157	0.131
GmU93	0.956	0.961	0.110	0.117	0.367	0.283
GmU95	0.136	0.171	0.040	0.166	0.270	0.125
GmU40	-0.028	0.024	0.051	-0.048	0.117	0.158
Mean	0.220	0.284	0.081	0.050	0.206	0.166
SE	0.166	0.147	0.020	0.037	0.041	0.024

near the sampling sites used in this study flow south to north, terminating into the Susquehanna. Previous research by Brown et al. (2016) indicated that Wood Turtles become more terrestrial as the thermoregulatory benefits of returning to the water at night diminish during the summer, but they never seem to stray too far from flowing water. Furthermore, a species of turtle that divides its time between land and water (Kaufmann 1992) is expected to use smaller rivers as corridors like many other turtle species (Gibbs and Amato 2000). If local Wood Turtles are using the Susquehanna River as a corridor at least in part, with strong flow and flooding events acting as a migration regulator, further genetic research should yield an F_{st} gradient and not complete differentiation. In other words, central New York's populations should be progressively more differentiated from populations further south along the Susquehanna River, but not completely differentiated altogether. Therefore, further research should investigate the potential of large rivers, namely the Susquehanna River, to act as turtle barriers or corridors.

If the Susquehanna is acting as a unidirectional barrier, long-term declines could prove problematic for the local populations. Specifically, the loss of only a few individuals may appear to be minimal in terms of allelic diversity, but a negative change in the effective breeding population (N_e), as was observed, could prove to have adverse conservation consequences. For example, similar rates of reduction in Bog Turtle populations have been identified as substantially increasing the likelihood of extirpation (Shoemaker 2011). Certain life histories are also known to be susceptible to such impacts (Jonsson and Ebenman 2001). Specifically, for Bog Turtle populations, the loss of only a few breeding adult individuals can have greater impacts on populations than even dramatic short-term increases in juvenile mortality (Shoemaker 2011). Similar losses for Wood Turtles, the closest known relative of the Bog Turtle, could prove to be equally problematic. This vulnerability, again, would be due to their long generation times, high juvenile mortality rate, and reliance on adult survival to bolster the populations (Gibbs and Amato 2000). This situation presents a suite of unique conservation issues which are likely to also be applicable to other freshwater turtle species.

One noteworthy observation here is that the bottleneck analysis presented identified both heterozygosity deficiency and excess in the northern populations. Typically, heterozygosity deficiency is associated with a founder effect (Cornuet and Luikart 1996) or possibly the existence of a subpopulation structure within the sample, known as the Wahlund effect (Wahlund 1928). However, in rare situations when allelic diversity is high, as it is with the Wood Turtles in this study, heterozygosity deficiency can be the result of post-bottleneck changes, such as mutation or population expansion, which fill the allelic gaps left by limited random selection (Cornuet and

Luikart 1996; Maruyama and Fuerst 1985). For freshwater turtle populations, which typically have a small number of long-lived adults possessing the majority of effective alleles (Crouse and Frazer 1995; Gibbs and Amato 2000), the loss and sequential replacement of these few valuable reproductive individuals appear to enable this particular scenario. Considering that none of the loci for the northern population were in mutation-drift equilibrium, and they showed evidence of deficiency and excess, it seems clear that something has impacted the northern population allelic ratios. Further research into Otsego County's populations north of Interstate 88 is required to determine the source of this irregularity. Additionally, we recommend that future management plans for Wood Turtle populations in central New York and other regions with montane-riverine mosaics consider the potential genetic complications associated with anthropogenic habitat fragmentation. To mitigate these potential impacts, the installation of appropriately sized culverts, drift nets, and turtle-crossing signs (Aresco 2005; Woltz et al. 2008), in high-density areas (Gunson and Schueler 2012), is necessary.

Conclusions

Consistent with other research (Steen and Gibbs 2004; Gibbs and Steen 2005; Steen et al. 2006) the observed division and reduction in N_e in the local populations studied here, the potential recent genetic bottleneck, the increased differentiation, and the overall change in population structure are most likely attributable to the additional fragmentation of the local montane/riverine habitat by the bisecting interstate highway. A clear north to south directionality of gene flow was observed from the short-term (25–125 years) migration estimate. The full implications of this dichotomy, in the context of potential isolation due to fragmentation, have yet to be determined. As a species of conservation concern, understanding the genetic landscape at the local and regional levels is vital for planning future management. In terms of conservation, it is possible New York's central populations hold unique alleles as they are surrounded by three major highways and two large mountain ranges. In turn, this may require that management efforts treat these isolated populations as demographically independent units, should they yield unique genetic variation. As such, we recommend that policy and management reflect the impacts that bisecting highways can have on populations within a local region, and not just those adjacent to a thoroughfare. Additionally, we recommend that policy and management efforts reflect the evidence, which suggests that hydrology may dictate Wood Turtle gene flow. Furthermore, research focused on determining where central New York's populations fit within the context of the entire region's genetic landscape will be particularly useful.

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Literature Cited

- Amato ML, Brooks RJ, Fu J. 2008. A phylogeographic analysis of populations of the Wood Turtle (*Glyptemys insculpta*) throughout its range. *Molecular Ecology* 17(2): 570–581.
- Associated Press. 1986. Albany firm bids low on I-88 work. *The Times Union (Albany, New York, USA)* 24 December 1986. Available: <http://tinyurl.com/gqrwh4p> [Accessed: 3 March 2017].
- Aresco MJ. 2005. Mitigation measures to reduce highway mortality of turtles and other herpetofauna at a north Florida lake. *Journal of Wildlife Management* 69(2): 549–560.
- Arthofer W, Heussler C, Krapf P, Schlick-Steiner BC, Steiner FM. 2018. Identifying the minimum number of microsatellite loci needed to assess population genetic structure: a case study in fly culturing. *Fly* 12(1): 13–22.
- Arvisais M, Lévesque E, Bourgeois JC, Daigle C, Masse D, Jutras J. 2004. Habitat selection by the Wood Turtle (*Clemmys insculpta*) at the northern limit of its range. *Canadian Journal of Zoology* 82(3): 391–398.
- Breisch AR, Behler JL. 2002. Turtles of New York State. *Conservationist* 57(3): 15–18.
- Brown DJ, Nelson MD, Rugg DJ, Buech RR, Donner DM. 2016. Spatial and temporal habitat use patterns of Wood Turtles at the western edge of their distribution. *Journal of Herpetology* 50(3): 347–356.
- Buhlmann KA, Gibbons JW. 1997. Imperiled aquatic reptiles of the southeastern United States: historical review and current conservation status. Pp. 201–232 In: *Aquatic Fauna in Peril: The Southeastern Perspective*. Editors, Benz GW, Collins DE. Special Publication 1, Southeast Aquatic Research Institute. Lenz Design and Communications, Decatur, Georgia, USA. 554 p.
- Castellano CM, Behler JL, Amato G. 2009. Genetic diversity and population genetic structure of the Wood Turtle (*Glyptemys insculpta*) at Delaware Water Gap National Recreation Area, USA. *Conservation Genetics* 10(6): 1,783–1,788.
- Chiucchi JE, Gibbs HL. 2010. Similarity of contemporary and historical gene flow among highly fragmented populations of an endangered rattlesnake. *Molecular Ecology* 19(24): 5,345–5,358.
- Cornuet JM, Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144(4): 2,001–2,014.
- Crouse DT, Frazer NB. 1995. Population models and structure. Pp. 601–603 In: *Biology and Conservation of Sea Turtles*. Editor, Bjorndal K. Smithsonian Institution Press, Washington, DC, USA. 620 p.
- Cureton JC, Janis M, Lutterschmidt WI, Randle CP, Ruthven DC, Deaton R. 2014. Effects of urbanization on genetic diversity, gene flow, and population structure in the Ornate Box Turtle (*Terrapene ornata*). *Amphibia-Reptilia* 35(1): 87–97.
- Edwardsen E. 1989. I-88 may be named after Anderson. *The Times Union (Albany, New York, USA)* 10 March 1989. Available: <http://tinyurl.com/zo6jgxw> [Accessed: 3 March 2017].
- Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14(8): 2,611–2,620.
- Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164(4): 1,567–1,587.
- Farrell RF, Graham TE. 1991. Ecological notes on the turtle *Clemmys insculpta* in northwestern New Jersey. *Journal of Herpetology* 25: 1–9.
- Galbraith DA, White BN, Brooks RJ, Kaufmann JH, Boag PT. 1995. DNA fingerprinting of turtles. *Journal of Herpetology* 29(2): 285–291.
- Galois P, Bonin J. 1999. *Rapport sur la Situation de la Tortue des Bois (Clemmys insculpta) au Québec*. Ministère de l'Environnement et de la Faune, Gouvernement du Québec, Québec, Canada. 62 p.
- Garber SD, Burger J. 1995. A 20-yr study documenting the relationship between turtle decline and human recreation. *Ecological Applications* 5(5): 1,151–1,162.
- Gibbons JW, Scott ED, Ryan TJ, Buhlmann KA, Tuberville TD, Metts BS, Greene JL, Mills T, Leiden Y, Poppy S, et al. 2000. The global decline of reptiles, déjà vu amphibians. *BioScience* 50(8): 653–666.
- Gibbs JP. 1993. Importance of small wetlands for the persistence of local populations of wetland-associated animals. *Wetlands* 13(1): 25–31.
- Gibbs JP. 2000. Wetland loss and biodiversity conservation. *Conservation Biology* 14(1): 314–317.
- Gibbs JP, Amato GD. 2000. Genetics and demography in turtle conservation. Pp. 207–217 In: *Turtle Conservation*. Editor, Klemens MW. Smithsonian

- Institution Press, Washington, DC, USA. 334 p.
- Gibbs JP, Shriver WG. 2002. Estimating the effects of road mortality on turtle populations. *Conservation Biology* 16(6): 1,647–1,652.
- Gibbs JP, Steen DA. 2005. Trends in sex ratios of turtles in the United States: implications of road mortality. *Conservation Biology* 19(2): 552–556.
- Gunson KE, Schueler FW. 2012. Effective placement of road mitigation using lessons learned from turtle crossing signs in Ontario. *Ecological Restoration* 30(4): 329–334.
- Hale ML, Burg TM, Steeves TE. 2012. Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. *PLoS One* 7(9): e45170.
- Harding JH, Bloomer TJ. 1979. The Wood Turtle, *Clemmys insculpta*, a natural history. *Bulletin of the New York Herpetological Society* 15(1): 9–26.
- Hartl DL, Clark AG. 1997. *Principles of Population Genetics*. Sinauer Associates, Sunderland, Massachusetts, USA. 635 p.
- Hulce D, Li X, Snyder-Leiby T, Liu CJ. 2011. GeneMarker® genotyping software: tools to increase the statistical power of DNA fragment analysis. *Journal of Biomolecular Techniques* 22(Suppl): S35–S36.
- Jones M, Sievert P. 2009. Effects of stochastic flood disturbance on adult Wood Turtles, *Glyptemys insculpta*, in Massachusetts. *Canadian Field Naturalist* 123(4): 313–322.
- Jones M, Willey L, Akre T, Seivert P. 2015. *Status and Conservation of the Wood Turtle (Glyptemys insculpta) in the Northeastern United States*. Technical Report for the Northeast Regional Conservation Needs Program. US Fish and Wildlife Service, Washington, DC, USA. 288 p.
- Jonsson A, Ebenman B. 2001. Are certain life histories particularly prone to local extinction? *Journal of Theoretical Biology* 209(4): 455–463.
- Kalinowski ST. 2002. How many alleles per locus should be used to estimate genetic distances? *Heredity* 88(1): 62.
- Kalinowski ST. 2005. Do polymorphic loci require large sample sizes to estimate genetic distances? *Heredity* 94(1): 33.
- Kammerer JC. 2005. *Largest Rivers in the United States*. United States Geological Service, Fairfax, Virginia, USA. Available: <https://pubs.usgs.gov/of/1987/ofr87-242/> [Accessed: 3 March 2017].
- Kaufmann JH. 1992. Habitat use by Wood Turtles in central Pennsylvania. *Journal of Herpetology* 26: 315–321.
- King TL, Julian SE. 2004. Conservation of microsatellite DNA flanking sequence across 13 emydid genera assayed with novel Bog Turtle (*Glyptemys muhlenbergii*) loci. *Conservation Genetics* 5(5): 719–725.
- Lamb T, Avise JC, Gibbons JW. 1989. Phylogeographic patterns in mitochondrial DNA of the Desert Tortoise (*Xerobates agassizi*), and evolutionary relationships among the North American gopher tortoises. *Evolution* 43(1): 76–87.
- Levell JP. 2000. Commercial exploitation of Blanding's Turtle, *Emydoidea blandingii*, and the Wood Turtle, *Clemmys insculpta*, for the live animal trade. *Chelonian Conservation and Biology* 3(4): 665–674.
- Lieberman S. 1994. Can CITES save the box turtle? *Endangered Species Technical Bulletin* 19(5): 1–16.
- Luikart G, Allendorf FW, Cornuet JM, Sherwin WB. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity* 89(3): 238–247.
- Maruyama T, Fuerst PA. 1985. Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics* 111(3): 675–689.
- Mills LS, Citta JJ, Lair KP, Schwartz MK, Tallmon DA. 2000. Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. *Ecological Applications* 10(1): 283–294.
- Peakall R, Smouse PE. 2006. GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6(1): 288–295.
- Peakall R, Smouse PE. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2,537–2,539.
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.
- Rhodin, AGJ, Walde AD, Horne BD, Van Dijk PP, Blanck T, Gudson R. 2011. Turtles in trouble: the world's 25+ most endangered tortoises and freshwater turtles-2001. *Turtle Conservation Coalition* 1: 1–58.
- Richtsmeier RJ, Bernstein NP, Demastes JW, Black RW. 2008. Migration, gene flow, and genetic diversity within and among Iowa populations of Ornate Box Turtles (*Terrapene ornata ornata*). *Chelonian Conservation and Biology* 7(1): 3–11.
- Sefc KM, Payne RB, Sorenson MD. 2003. Microsatellite amplification from museum feather samples: effects of fragment size and template concentration on genotyping errors. *The Auk* 120(4): 982–989.
- Shoemaker KT. 2011. Demography and population genetics of the Bog Turtle (*Glyptemys muhlenbergii*): implications for regional conservation planning in New York State. Ph.D. Dissertation, State University of New York College of Environmental Science and Forestry of New York, Department of Environmental Forest Biology, Syracuse, New York, USA. 210 p.
- Spradling TA, Tamplin JW, Dow SS, Meyer KJ. 2010. Conservation genetics of a peripherally isolated

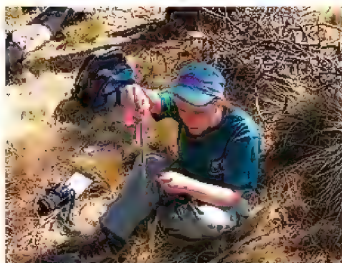
- population of the Wood Turtle (*Glyptemys insculpta*) in Iowa. *Conservation Genetics* 11(5): 1,667–1,677.
- Steen DA, Aresco MJ, Beilke SG, Compton BW, Condon EP, Dodd KC, Forrester H, Gibbons JW, Greene JL, Johnson G, et al. 2006. Relative vulnerability of female turtles to road mortality. *Animal Conservation* 9(3): 269–273.
- Steen DA, Gibbs JP. 2004. Effects of roads on the structure of freshwater turtle populations. *Conservation Biology* 18(4): 1,143–1,148.
- Sweeten SE. 2008. Home range, hibernacula fidelity, and best management practices for Wood Turtles (*Glyptemys insculpta*) in Virginia. M.S. Thesis, James Madison University of Virginia, Department of Biology, Harrisonburg, Virginia, USA. 99 p.
- Tessier N, Paquette SR, Lapointe FJ. 2005. Conservation genetics of the Wood Turtle (*Glyptemys insculpta*) in Quebec, Canada. *Canadian Journal Zoology* 83(6): 765–772.
- Tuttle SE, Carroll DE. 2003. Home range and seasonal movements of the Wood Turtle (*Glyptemys insculpta*) in southern New Hampshire. *Chelonian Conservation and Biology* 4(3): 656–663.
- US Fish and Wildlife Service. 2015. Species profile for Wood Turtle (*Glyptemys insculpta*). Available: <http://ecos.fws.gov/ecp0/profile/speciesProfile?spcode=C06A> [Accessed: 3 March 2017].
- Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4(3): 535–538.
- Wahlund S. 1928. Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. *Hereditas* 11(1): 65–106.
- Williams T. 1999. The terrible turtle trade: the pet trade is decimating turtle populations and spreading disease. *Audubon* 101: 44–51.
- Wilson GA, Rannala B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* 163(3): 1,177–1,191.
- Woltz HW, Gibbs JP, Ducey PK. 2008. Road crossing structures for amphibians and reptiles: informing design through behavioral analysis. *Biological Conservation* 141(11): 2,745–2,750.
- Wood RC, Herlands R. 1997. Turtles and tires: the impact of roadkills on Northern Diamondback Terrapin, *Malaclemys terrapin terrapin*, populations on the Cape May Peninsula, Southern New Jersey, USA. Pp. 46–53 In: *Proceedings, Conservation, Restoration, and Management of Tortoises and Turtles: an International Conference: 11–16 July 1993, State University of New York, Purchase, New York, USA, Volume 1*. New York Turtle and Tortoise Society, Bronx, New York, USA. 494 p.



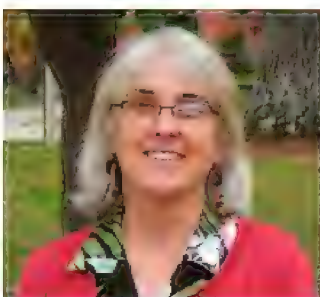
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Modelling the distribution of the Ocellated Lizard in France: implications for conservation

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Abstract.—The Ocellated Lizard, *Timon lepidus* (Daudin 1802) occupies the Mediterranean regions of southwestern Europe (Portugal, Spain, France, and the extreme northwest of Italy). Over the last decades, a marked decline in its population has been observed, particularly on the northern edge of its distribution. As a result, it is currently considered a threatened species, especially in France and Italy. In France, a national action plan for its conservation has been put in place. In this study, ecological niche modelling (ENM) was carried out over the entire area of France in order to evaluate the species' potential distribution, more accurately define its ecological niche, guide future surveys, and inform land use planning so this species can be better taken into consideration. The modelling used data representing 2,757 observation points spread over the known range of the species, and 34 ecogeographical variables (climate, topography, and vegetation cover) were evaluated. After removing correlated variables, models were fitted with several combinations of variables using eight species distribution model (SDM) algorithms, and then their performance was assessed using three model accuracy metrics. Iterative trials changing the input variables were used to obtain the best model. The optimized model included nine determining variables. The results indicate the presence of this species is linked primarily to three climate variables: precipitation in the driest month, precipitation seasonality, and mean temperature in the driest quarter. The model was checked by a sample dataset that was not used to fit the model, and this validation dataset represented 25% of the overall field observations. Of the known occurrence locations kept aside to check the results, 94% fell within the presence area predicted by the modelled map with a presence probability greater than 0.7, and 90% fell within the area with a presence probability ranging from 0.8 to 1, which represents a very high predictive value. These results indicate that the models closely matched the observed distribution, suggesting a low impact of either geographical factors (barriers to dispersal), historical factors (dispersal process), or ecological factors (e.g., competition, trophic resources). The overlap between the predicted distribution and protected areas for this species reveals that less than 1% of the potential distribution area is protected by strong regulatory measures (e.g., national parks and natural reserves). The knowledge obtained in this study allows us to recommend some guidelines that would favor the conservation of this species.

Résumé.—Le lézard ocellé, *Timon lepidus* (Daudin 1802), occupe les régions méditerranéennes du sud-ouest de l'Europe (Portugal, Espagne, France, et extrême nord-ouest de l'Italie). Au cours des dernières décennies, un fort déclin des populations a été observé, particulièrement aux marges nord de sa distribution. Il est donc considéré comme une espèce menacée, spécialement en France et en Italie. En France, il bénéficie d'un plan national d'actions en faveur de sa préservation. La modélisation de sa distribution a été conduite sur l'ensemble du territoire national en vue d'estimer sa distribution potentielle, préciser sa niche écologique, orienter les prospections futures et permettre une meilleure prise en compte de l'espèce dans l'aménagement du territoire. Le travail de modélisation repose sur 2757 points d'observation répartis sur l'ensemble de la distribution connue de l'espèce, confrontés à 34 variables climatiques, topographiques, et de couvert végétal. Après suppression des variables autocorrélées, plusieurs combinaisons de variables ont été testées, et leur performances évaluées à partir de huit algorithmes SDM. Le meilleur modèle retient neuf variables, déterminées par l'algorithme ayant la meilleure performance. Les modèles montrent que la présence de l'espèce est

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principalement déterminée par la sécheresse et la température estivale: précipitations au cours du mois le mois le plus sec, saisonnalité des précipitations et température moyenne des trois mois les plus chauds. La validation du modèle sur la base d'un échantillon totalisant 25 % du total des observations, non inclus dans le modèle, montre que 94 % des données de validation se placent dans l'aire potentielle au seuil de probabilité de 0,7, et 90 % pour une probabilité comprise entre 0,8 et 1. Ceci donne une valeur prédictive très élevée au modèle retenu. On constate une étroite concordance entre la distribution potentielle et la distribution réalisée, ce qui suggère une faible influence des facteurs géographiques (obstacles à la dispersion), historiques (processus de dispersion) ou écologiques (compétition, ressources trophiques, etc.). Le croisement cartographique entre l'aire potentielle de l'espèce et les espaces protégés montre que moins de 1 % de l'aire potentielle est couverte par des mesures réglementaires fortes (parcs nationaux et réserves naturelles). En conclusion, le travail donne des orientations pour améliorer la connaissance de la distribution de l'espèce et des pistes de réflexion en faveur de sa conservation.

Keywords. *Timon lepidus*, species distribution models, ecological niche, Europe, Reptilia, Sauria, Squamata

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Introduction

The success of conservation programs in protecting threatened species depends largely on the quality of the information related to the environmental conditions favorable to (or sought by) the species (Griffith et al. 1989; Souter et al. 2007; Fourcade et al. 2018). For this reason, ecological (or environmental) niche models (ENMs) [Sillero 2011], also known as species distribution models (SDMs) or habitat distribution models (HDMs), are increasingly used to inform conservation measures (Ferrier 2002; Graham et al. 2004; Araújo and Segurado 2004; Santos et al. 2009; Elith and Leathwick 2009; Lyet et al. 2013; Jiang et al. 2014; Wan et al. 2016; Ianella et al. 2018). These models allow researchers to identify the factors which explain the distribution of a species (Austin et al. 1990; Vetaas 2002; Guisan and Hofer 2003; Jiang et al. 2014; Ferreira et al. 2013), to orient research toward zones where the species has not yet been identified (Engler 2004; Raxworthy et al. 2003; Lyet et al. 2013; GHRA-LPO Rhône-Alpes 2015; Ryberg et al. 2017), to identify the most favorable zones for the conservation of the species (Brito et al. 1996; Barbosa et al. 2003; Anderson and Martinez-Meyer 2004; Muñoz et al. 2005; Guisan et al. 2013; Lyet et al. 2013; Wang et al. 2016; Moradi et al. 2019; Sohrab et al. 2019), to evaluate the potential dispersion and gene flow between population patches (Guisan and Thuiller 2005), to model future changes in distribution (for example, based on climate change) [Franklin 1998; Guisan and Hofer 2003; Araújo et al. 2006; Carvalho et al. 2011; Cheaib et al. 2012; Ianella et al. 2018; Renwick et al. 2018], as well as to project the distribution into past scenarios (Sillero and Carretero 2013).

Species distribution models also allow comparisons

between potential and actual distributions, two very useful concepts to consider in conservation biology. Put simply (see Pulliam 2000 for a more detailed explanation), a potential niche corresponds to areas in which the climatic, terrain, and habitat conditions are theoretically favorable to the species in the current conditions, whereas the realized niche takes into account historical and biotic factors that may explain the absence of the species within the ecological area defined by the fundamental niche. Comparing these two niches provides information about the historical processes that led to the current distribution, the dispersion capacity of the species, and the obstacles to its dispersion. This can illuminate the ecological factors that negatively influence the presence of the species, such as the presence of competitors or predators, unsuitable habitats, insufficient trophic resources, a lack of host species, and others (see Guisan and Thuiller 2005). When used to model the distribution of a species in decline, SDMs can also provide information about the causes of decline, particularly by helping to differentiate the proportions due to global factors as opposed to regional or local factors (Jiang et al. 2014). In some cases, SDMs can even allow the extent of decline to be measured, by comparing the potential niche with the observed niche (Lyet et al. 2013; Ryberg et al. 2017).

The Ocellated Lizard, *Timon lepidus* (Daudin 1802), is a good case study for this type of analysis. This species occupies the Mediterranean regions of southwestern Europe (Portugal, Spain, France, and the extreme northwest of Italy) [Figs. 1 and 2]. At the edges of its distribution, it has faced a marked population decline over the last decades and is now considered a threatened species, especially in France and Italy (Salvidio et al. 2004; Cheylan and Grillet 2005; Cheylan 2016).

This species is closely linked to the Mediterranean



Fig. 1. *Timon lepidus*, adult male, Hérault, France. Photo by Jean Nicolas.

climate and specific biotopes (Doré et al. 2015). Moreover, due to its large size and thermal requirements for reproduction, it is particularly demanding regarding climatic conditions (Mateo 2011). The incubation period for its eggs is long, about 100 days, making use of the full period of warmer temperatures. The eggs hatch late, generally at the end of September or the beginning of October (Bischoff et al. 1984; Doré et al. 2015). Hence, two main factors drive the distribution of this species: its ecophysiology (thermal requirements linked to the individual's bulk and to the incubation of the eggs) and its habitat requirements (dry habitats with little tree cover). In view of these needs, the Ocellated Lizard should theoretically benefit from the warming temperatures recorded in Europe over the last 30 years (see Prodon et al. 2017). Yet observations show a rather widespread decline of this species, particularly on the northern edge of its distribution range, which is inconsistent with the expected situation in warming conditions (Salvidio et al. 2004; Cheylan and Grillet 2005; Doré et al. 2015). This raises questions regarding the causes of the decline of this species and, at first glance, suggests a hypothesis that local effects predominate over global effects.

The use of SDMs allows the study of interesting biogeographical questions about this species. Native to the Iberian Peninsula, the Ocellated Lizard colonized France and the extreme west of Italy along the Mediterranean coast (Sillero et al. 2014; Doré et al. 2015). This colonization involved overcoming considerable physical obstacles, including rivers, mountains, and forests. Niche modelling can provide information about constraints that limit dispersion; that is, whether the current distribution boundaries of the species are of a climatic nature (thus ecophysiological) or a physical nature (due to obstacles to dispersion). The same question applies to the species' colonization of the French Atlantic coast: Are the isolated populations along this coast the result of a process of decline linked to the progressive degradation of habitat or to climatic constraints? The responses to these questions can be found by comparing the expected distribu-



Fig. 2. *Timon lepidus*, juvenile, Hérault, France. Photo by Jean Nicolas.

tion with the observed distribution; that is, by comparing the potential niche with the realized niche.

This study used species distribution modelling to investigate the following questions: (1) Does the observed distribution of the Ocellated Lizard match its potential distribution? If not, why not? (2) Which variables best explain the distribution of this species: climate, terrain, land use, or other factors? (3) Why is this species retreating at the edges of its distribution range, in contrast to what might be expected based on climatic changes? (4) Are the distribution boundaries of this species conditional on either climate, physical barriers, or ecological causes? (5) Which zones are potentially the most favorable for the conservation of this species? (6) Where should future surveys be carried out to improve our understanding of the distribution range? (7) Based on these findings, what conservation strategy should be implemented for the conservation of the species?

Materials and Methods

Data

Ocellated Lizard dataset. This study used observations (presence data only, Brotons et al. 2004) from an exhaustive database that includes most of the occurrences observed in France between 1970 and 2016. As the objective was essentially practical, i.e., to identify the areas of the potential presence of the species with the aim of its conservation, this study did not consider taking into account the entire distribution of the species as relevant to building the model. This would have led to further complications, such as the need to consider markedly divergent genetic lines and, as a result, ecophysiological adaptations specific to the regions that host these genetic lines.

The data were collected by a number of organizations and individuals over a period of 46 years. Before integrating the data into the models, the records were verified and cross-validated, keeping only precisely georefer-

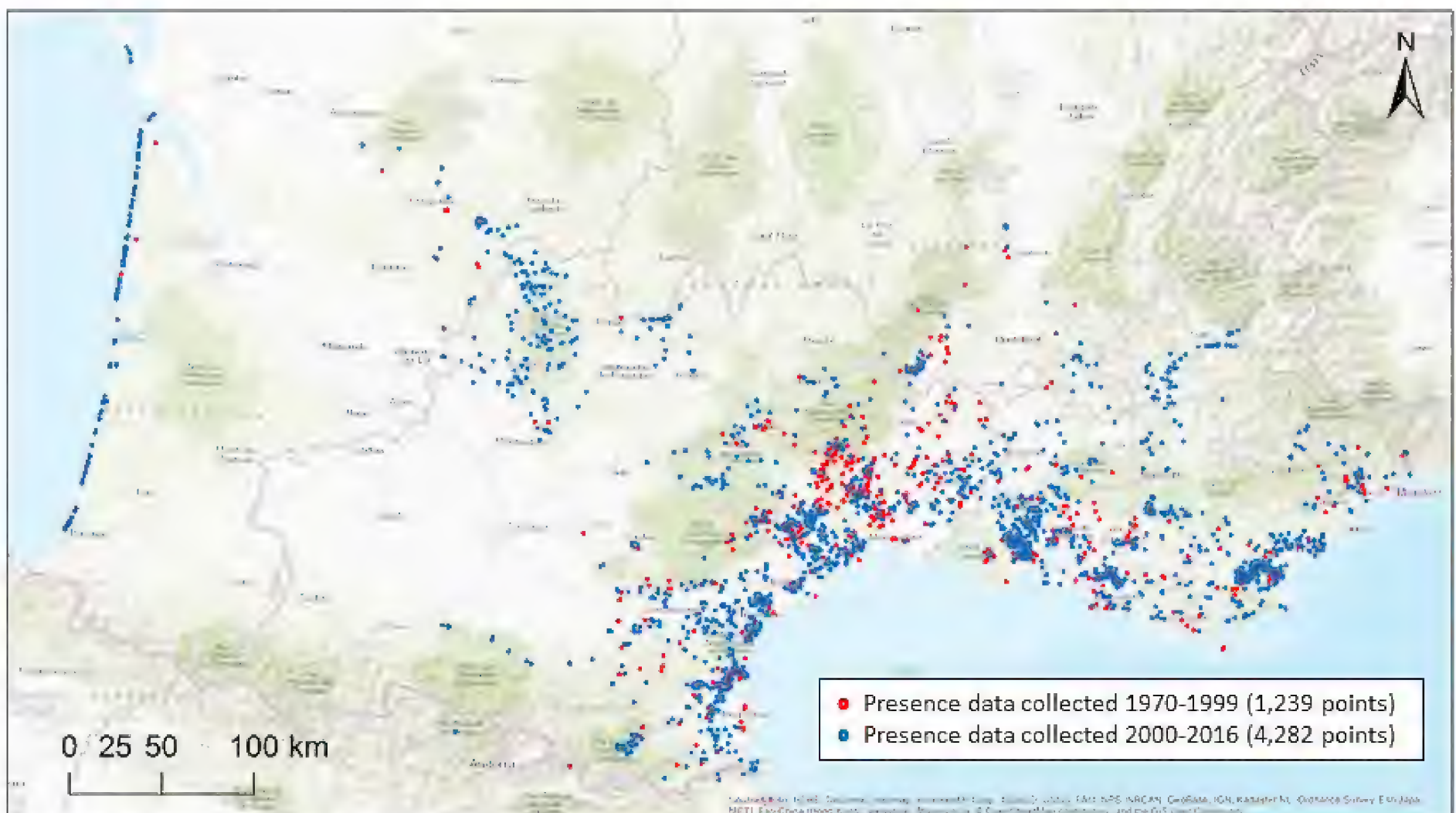


Fig. 3. Localization of the total presence data for the Ocellated Lizard *Timon lepidus* collected in France during the period 1970 to 2016.

enced locations (either data captured by GPS or observations recorded with a spatial positioning error of less than 100 m). This resulted in presence data for a total of 5,521 locations spread over southern France. From this, a sample dataset was extracted for modelling purposes. First, only occurrences for the period that corresponded to the vegetation variable used in the model were selected, taking into account the development and continuity of the vegetation cover over the years. To fit this requirement, the presence data were narrowed down to a sourcing period of 16 years, which included 4,282 observations from 2000 to 2016. The distribution of occurrence data for this period covers the whole area of study, though data prior to the year 2000 were not included (Fig. 3).

Secondly, the dataset was filtered to avoid spatial bias due to oversampling at particular locations, as field investigations conducted for environmental impact assessments and other monitoring programs led to a higher concentration of data at certain sites. Therefore, the density of points per km² was evaluated to identify zones subject to sampling bias (Fig. 1 in Supplementary Materials), using kernel density calculation. Through this analysis, zones where the point density ranged from 2–50 points per km² were determined, and for these zones, a single record was retained per km². After filtering the data by density, a total of 2,757 occurrences were retained, providing presence data that was well distributed over the study area (Fig. 4). Finally, of these 2,757 valid records, 75% were randomly selected for modelling, with the remaining 25% serving as an independent source to check (validate) the results (Hirzel and Guisan 2002) [Fig. 4]. During the modelling procedure, the database consisting

of 75% of the occurrences was itself divided into training data (80%) and testing data (20%).

Environmental data. A set of ecogeographical variables was used to model species distribution, taking into account the ecological requirements of the species (Guisan and Thuiller 2005). A group of 34 variables was evaluated through several iterations in order to identify and include the most relevant variables (Table 1).

As a first step, environmental variables were obtained from available sources at the appropriate spatial and thematic resolutions. This study used the CHELSA database, version 1.1 (Climatologies at High Resolution for the Earth's Land Surface Areas, Karger et al. 2017). This database includes a set of bioclimatic variables, with monthly mean temperature and precipitation patterns, for the time period 1979–2013, which corresponds to the time range of the species occurrence data. The CHELSA database is an alternative source to the widely used WorldClim global climate database, as both derive their bioclimatic variables from the monthly minimum, maximum, and mean temperatures, as well as precipitation values. However, as described by Karger et al. (2017), the CHELSA variables include additional corrections, such as monthly mean and station bias, wind effect and valley exposition, as well as correction for orographic effects on precipitation. Additionally, as CHELSA is a recently released product, this study allowed evaluation of its potential for species distribution modelling. In a recent study, Karger et al. (2017) highlighted differences observed at a large scale between WorldClim and CHELSA models, with the latter leading to a significant

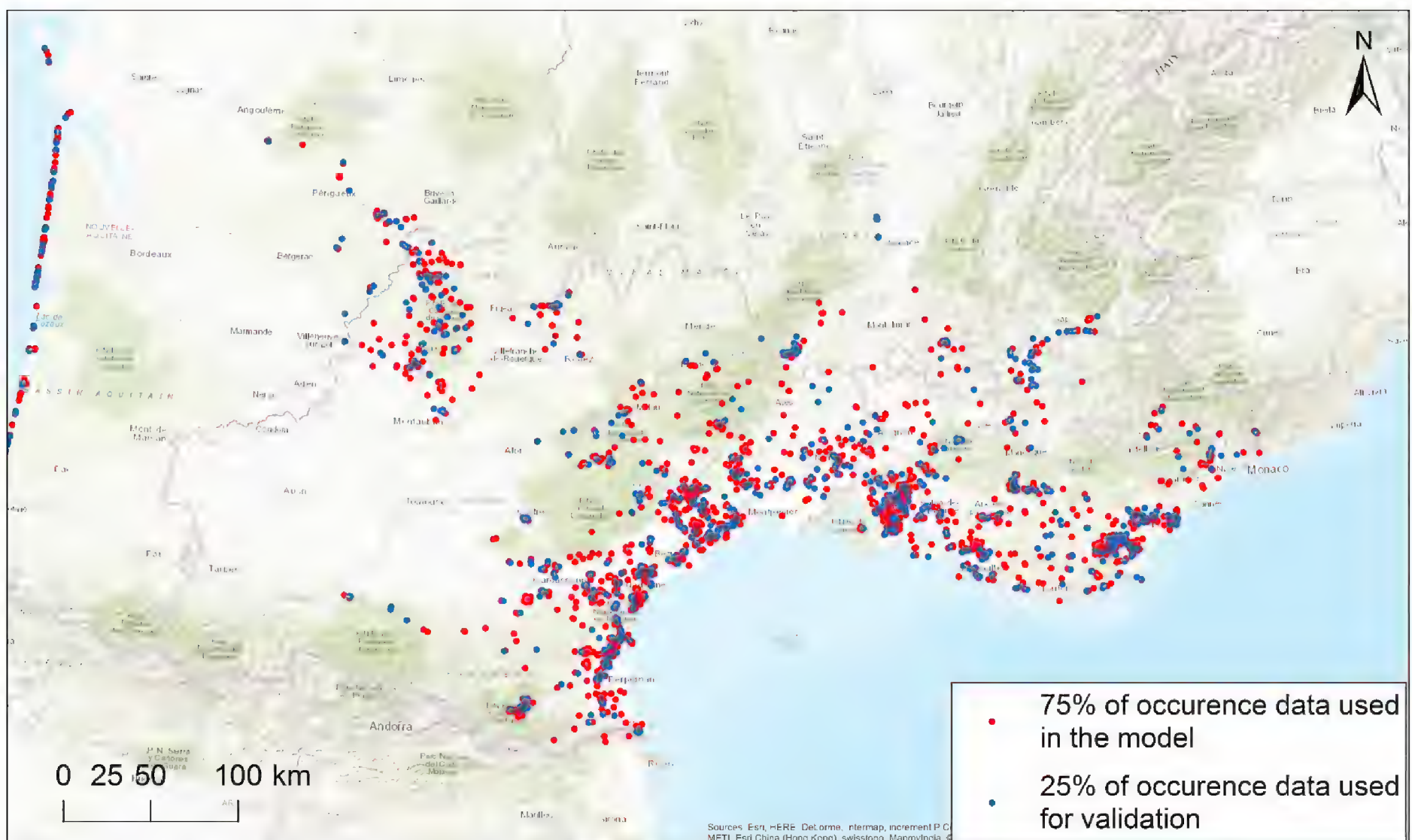


Fig. 4. Localization of occurrence data for the Ocellated Lizard *Timon lepidus*, showing the points in the dataset used for the model (red) and those used to check the model (blue).

improvement in SDM performance. The CHELSA bioclim data grid has a 30-arc-second pixel resolution, corresponding to a spatial resolution of 0.00833 decimal degrees at the equator. Applied to the study area in southern France, the bioclimatic variables have a spatial resolution of less than 1 km², with each grid roughly covering an area of 700 x 900 m.

As the CHELSA database does not include variables reflecting solar radiation and its impact on climatic humidity or aridity, data produced by the Consultative Group on International Agricultural Research (CGIAR) were used (Fick and Hijmans 2017). Data available at the same resolution as the CHELSA dataset included mean annual solar radiation, potential evapotranspiration, and a global aridity index calculated from the ratio of mean annual precipitation to mean annual potential evapotranspiration.

During the process of selecting and consolidating the ecogeographical variables, another climate data source was used to compare the results and identify the best input that would optimize performance. This climate dataset (Joly et al. 2010) is based on data from Météo France's weather stations and gathers a number of indicators that are not provided in the CHELSA dataset, such as the number of days with a temperature above 30°C or below -5 °C, as well as other parameters related to temperature ranges and seasonal variations over the year.

To increase model performance, topographic data were also included. The elevation was obtained from the EU-DEM v1.1 dataset, produced in the framework of the European Commission's Copernicus program (Bashfield and Keim 2011), which includes a digital elevation

model captured in 2011 and projected in ETRS89-LAEA (EPSG code 3035), with a spatial resolution of 25 m. This allowed the modelling to take altitude into account, as well as slope and aspect, calculated in projected coordinate systems (all in meters).

A variable representing vegetation cover is also required, in order to identify natural habitats suitable for the Ocellated Lizard. The vegetation cover indicator selected was the normalized difference vegetation index (NDVI) generated from the MODIS (Moderate resolution Imaging Spectro-radiometer) Terra satellite sensors (Huete et al. 2010), which has a spatial resolution of 250 m. Data captured in the first week of July were used, as this corresponds to the optimal season for identifying the permanent vegetation cover of interest in this study. Vegetation indices in summer can highlight chlorophyll produced by permanent vegetation, whereas vegetation indices calculated in spring are influenced by the annual growth of the herbaceous stratum.

Considering the possibility that land cover has changed over time, the importance of changes in vegetation cover over the years was evaluated. The NDVI values were compared for each year from 2000 to 2016, calculating the variance and the standard deviation of the mean NDVI value for this 16-year period (Fig. 5). The results indicated that the change in permanent vegetation cover in the area of interest has been negligible, especially in areas where the Ocellated Lizard has been observed. Over 16 years, for the month of July, the standard deviation of the yearly NDVI values relative to the NDVI mean value was below 0.075 for 98.9% of the entire studied area, below 0.05 for 88.9% of the area, and

Table 1. Description of the 34 variables evaluated.

Variable	Description	Source	Period	Resolution
Bio1	Annual mean temperature	CHELSA v1.1	1979–2013	~1 km ²
Bio3	Isothermality (BIO2/BIO7) (*100)	CHELSA v1.1	1979–2013	~1 km ²
Bio4	Temperature seasonality (standard deviation *100)	CHELSA v1.1	1979–2013	~1 km ²
Bio5	Max temperature in warmest month	CHELSA v1.1	1979–2013	~1 km ²
Bio8	Mean temperature in wettest quarter	CHELSA v1.1	1979–2013	~1 km ²
Bio9	Mean temperature in driest quarter	CHELSA v1.1	1979–2013	~1 km ²
Bio10	Mean temperature in warmest quarter	CHELSA v1.1	1979–2013	~1 km ²
Bio13	Precipitation in wettest month	CHELSA v1.1	1979–2013	~1 km ²
Bio14	Precipitation in driest month	CHELSA v1.1	1979–2013	~1 km ²
Bio15	Precipitation seasonality (coefficient of variation)	CHELSA v1.1	1979–2013	~1 km ²
Bio16	Precipitation in wettest quarter	CHELSA v1.1	1979–2013	~1 km ²
Bio17	Precipitation in driest quarter	CHELSA v1.1	1979–2013	~1 km ²
Bio 18	Precipitation in warmest quarter	CHELSA v1.1	1979–2013	~1 km ²
Bio19	Precipitation in coldest quarter	CHELSA v1.1	1979–2013	~1 km ²
Alt	Elevation	EU-DEM v1.0 European Commission Copernicus program	2011	25 m
Slope	Slope	Calculated from EU-DEM v1.0	2011	25 m
Aspect	Slope orientation	Calculated from EU-DEM v1.0	2011	25 m
NDVI	Normalized vegetation index	MODIS TERRA (NASA)	July 2012	250 m
TCD	Tree cover density	Copernicus	2012	20 m
srad	Mean annual solar radiation	WORLDCLIM v.2	1970–2000	~1 km ²
PET	Global potential evapotranspiration	CGIAR	1950–2000	~1 km ²
Aridity	Global aridity index (mean annual precipitation / mean annual PET)	CGIAR	1950–2000	~1 km ²
TMO	Annual mean temperature	ThéMA - CNRS-UMR 6049	1971–2000	250 m
TMN	Number of days with temperature below -5 °C	ThéMA - CNRS-UMR 6049	1971–2000	250 m
TMX	Number of days with temperature above 30 °C	ThéMA - CNRS-UMR 6049	1971–2000	250 m
TAM	Annual temperature range	ThéMA - CNRS-UMR 6049	1971–2000	250 m
TEH	Inter-annual temperature variability in January	ThéMA - CNRS-UMR 6049	1971–2000	250 m
TEE	Inter-annual temperature variability in July	ThéMA - CNRS-UMR 6049	1971–2000	250 m
PDH	Variance between January precipitation and monthly mean precipitation	ThéMA - CNRS-UMR 6049	1971–2000	250 m
PDE	Variance between July precipitation and monthly mean precipitation	ThéMA - CNRS-UMR 6049	1971–2000	250 m
PJH	Number of rainy days in January	ThéMA - CNRS-UMR 6049	1971–2000	250 m
PEH	Inter-annual precipitation variability in January	ThéMA - CNRS-UMR 6049	1971–2000	250 m
PEE	Inter-annual precipitation variability in July	ThéMA - CNRS-UMR 6049	1971–2000	250 m
PRA	Variation between autumn (September and October) and July precipitation	ThéMA - CNRS-UMR 6049	1971–2000	250 m

only reached a maximum of 0.2 for 1.1% of the area. An assessment of the changes in vegetation cover within the study area allowed the selection of the NDVI mean value for the 16-year NDVI dataset (from 2000 to 2016).

All the selected variables were aggregated to a spatial resolution of ~1 km, using bilinear resampling techniques.

Methodology

The performance of models was explored based on different combinations of ecogeographical variables using an iterative approach (Heikkinen et al. 2006). To do this, a model was first fitted with a selected set of variables (Bucklin et al. 2014) and its performance was measured.

Table 2. Input variables for the six models. See Table 1 for descriptions of variables.

Code	Model description	Variables
M1	six selected bioclimatic variables	Bio4, Bio9, Bio10, Bio14, Bio15, Bio16
M2	eight random bioclimatic variables (blind test)	Bio3, Bio4, Bio5, Bio8, Bio9, Bio13, Bio17, Bio19
M3	12 climatic variables from Météo France	TMO, TMN, TMX, TAM, TEH, TEE, PDH, PDE, PJH, PEH, PEE, PRA
M4	M1 variables + solar radiation + PET + aridity	[Bio1, Bio4, Bio9Bio15, Bio16] + srad + PET + Aridity
M5	M1 variables + altitude, slope, aspect	[Bio4, Bio9, Bio10, Bio14, Bio15, Bio16] + Alt, Slope, Aspect
M6	M1 variables + altitude, aspect + vegetation	[Bio4, Bio9, Bio10, Bio14, Bio15, Bio16] + [Alt, Aspect] + NDVI

The inputs were then optimized by testing the model with other sets of variables, using a stepwise procedure to include or exclude variables one by one. Every iteration was evaluated and outcomes associated with the chosen parameters were recorded. This resulted in six models based on different combinations of variables (Table 2).

While a comparison of bioclimatic data sources in terms of usefulness for species distribution modelling is beyond the scope of this study, a deliberate choice was made to use various sources in the model; specifically, bioclimatic data from entirely different inputs. This iterative and multiple-source approach increases the chances of obtaining a successful model by allowing the selection

of the most accurate and relevant variables. It also helps to validate overall model performance, by providing keys for analyzing the suitability of the model in terms of bioclimatic variables favorable to the species.

Model descriptions

Model 1: Variables selected from the CHELSA climate dataset based on ecological assumptions. The first modelling trial used a set of ecogeographical variables identified based on expert knowledge of the biology and behavior of the species. As the Ocellated Lizard is a Mediterranean species that prefers long periods of warm

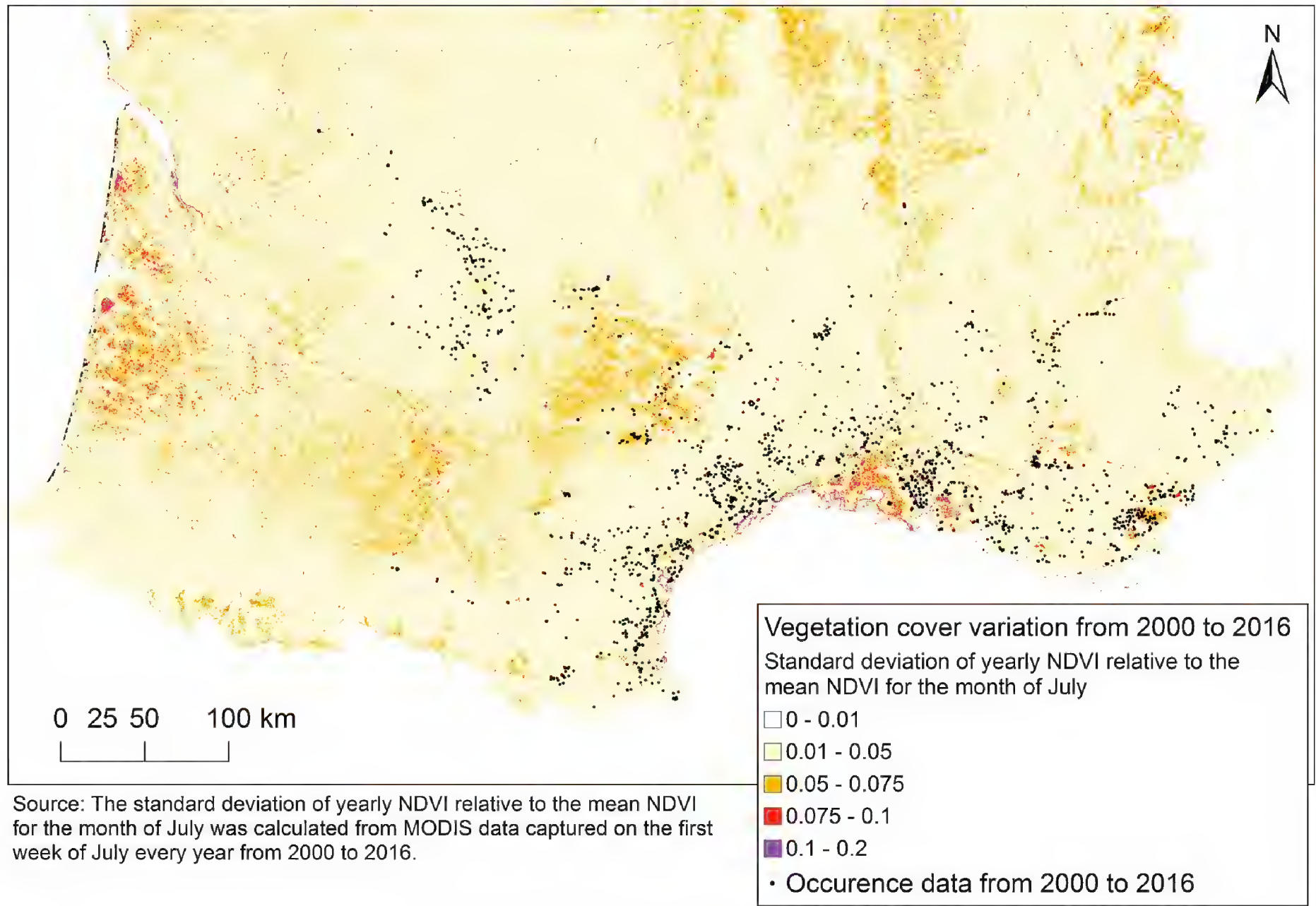


Fig. 5. Change in vegetation cover from 2000 to 2016: standard deviation of the yearly NDVI values relative to the NDVI mean value for the month of July.

Table 3. Variable correlation values for Model 1.

	bio4_R	bio9_R	bio10_R	bio14_R	bio15_R	bio16_R
bio4_R	1.00000000	-0.07440553	0.3081616	-0.2788248	0.1672821	-0.2605788
bio9_R	-0.07440553	1.00000000	0.5115825	-0.4025089	0.3664137	-0.0604624
bio10_R	0.30816159	0.51158248	1.0000000	-0.6782349	0.6210210	-0.1046921
bio14_R	-0.27882481	-0.40250891	-0.6782349	1.0000000	-0.6896546	0.2434781
bio15_R	0.16728213	0.36641372	0.6210210	-0.6896546	1.0000000	0.2159100
bio16_R	-0.26057884	-0.06046240	-0.1046921	0.2434781	0.2159100	1.0000000

temperatures (for ecological reasons linked to its reproductive cycle), this model considered the bioclimatic variables that would best represent this need. Variables with a recognized influence on a species' ecology are generally expected to lead to more accurate predictions in SDMs (Austin 2002). This initial SDM incorporated the following variables from the CHELSA database: Bio4, Bio9, Bio10, Bio14, Bio15, and Bio16 (Table 3). The Bio16 variable was selected as it represents precipitation in the wettest quarter, so it would be a good indicator of the aridity of the environment. This variable differentiates regions according to precipitation patterns by indicating rainfall occurring during the wettest period of the year, thus allowing locations with lower rainfall to be identified. After this *a priori* selection, the correlations between variables were measured (Table 3). As the variables were not correlated, this choice was validated.

Model 2: Non-correlated variables selected from the overall CHELSA climate dataset. For the next model, the correlations between the 19 variables obtained from the original CHELSA database for all presence-data locations were calculated, removing one of each pair of highly correlated variables (those with a correlation coefficient greater than 0.75) [Doorman 2012]. As 11 of the 19 input variables were correlated, only the remaining eight were retained, with no consideration of any presumed ecological significance. Thus, this trial was considered a blind test, run on a statistical basis only rather than on prior knowledge of the input variables. Problems of collinearity between variables were identified and dealt with using variance inflation factors (VIF) with the R usdm package (Naimi et al. 2014). A VIF was calculated for each explanatory variable, and those with a VIF greater than 10 were removed. The correlation coefficients of the remaining variables ranged between -0.015 and 0.75 (Table 1 in Supplementary Materials).

Model 3: Non-correlated variables from the Météo France climate dataset. As an alternative to the CHELSA climate database, this model used a climate dataset obtained from Météo France weather stations (Joly et al. 2010). Correlation tests showed that out of 14 variables from the Météo France dataset, only two were highly correlated, with a correlation coefficient greater than 0.75. Therefore, the 12 non-correlated variables were included in the model, with each having a potential effect on model performance.

Model 4: The variables for Model 1 with the addition of solar radiation, evapotranspiration, and aridity. This model included the six climate variables selected for Model 1 in addition to climatic parameters that are potentially important to the ecology of the species. To reflect discriminating factors related to the Mediterranean climate, this model used mean annual solar radiation (obtained from WorldClim), as well as the Global Potential Evapotranspiration and Global Aridity Index (obtained from CGIAR) [Fick and Hijmans 2017]. The Global Aridity Index consists of mean annual precipitation divided by mean annual potential evapotranspiration (Zomer et al. 2008). An assessment of whether the performance of the model increased with these additional variables was made.

Model 5: The variables for Model 1 with the addition of topographic variables. This model included the six climate variables selected for Model 1 along with three additional topographic variables: elevation, slope, and aspect (orientation). Topographic parameters were expected to improve model performance (Humboldt and Bonpland 1805).

Model 6: The variables for Model 1 with the addition of selected topographic variables and a vegetation variable. The final model included the six climate variables selected for Model 1 along with two additional topographic variables (elevation and aspect) as well as NDVI. The two topographic parameters were retained because of the gain in performance obtained by adding them to the bioclimatic parameters. The addition of the NDVI helped to account for vegetation cover as a contributing variable in the model, as this is a valuable parameter in identifying the natural habitat of the species. Assuming the distribution of the Ocellated Lizard is linked to this species' preferences in terms of land cover, vegetation density is expected to help differentiate areas of occurrence from areas of absence (Wilson et al. 2013).

SDM methods

To maximize SDM accuracy, all six models were run with eight statistical algorithms (Bucklin et al. 2014), regression-based machine learning, and classification methods. With each algorithm resulting in different pre-

Table 4. Algorithms used in the species distribution modelling.

Code	Description
BRT	Boosted Regression Trees
CART	Classification and Regression Trees for Machine Learning
GAM	Generalized Additive Model
GLM	Generalized Linear Model
MARS	Multivariate Adaptive Regression Spline
MAXLIKE	Maximum Likelihood
RF	Random Forests
SVM	Support Vector Machine

dictions, the objective was to identify the method that achieved the best accuracy (Elith et al. 2006). Testing eight algorithms also allowed the evaluation of the overall modelling approach (Table 4). As well as analyzing discrepancies between models in terms of performance, model congruence was examined to consolidate the conceptual approach (Li and Wang 2012). The modelling methods used were Generalized Linear Modelling (GLM, Guisan and Zimmerman 2000), Generalized Additive Modelling (GAM, Guisan and Zimmerman 2000), Multivariate Adaptive Regression Spline (MARS, Elith and Leathwick 2007), Maximum Likelihood (MAXLIKE), Classification and Regression Trees for Machine Learning (CART, Breiman et al. 1984), Boosted Regression Trees (BRT, Elith et al. 2008), Support Vector Machine (SVM, Drake et al. 2006), and Random Forests (RF, Breiman 2001).

The presence-absence models were used with the objective of predicting the presence probability of the Ocel-lated Lizard and mapping its distribution accordingly. Lacking absence data, pseudo-absences were used to run the models. Pseudo-absence data was generated with the R sdm package (Naimi and Araújo 2016), which has the advantage of providing a pseudo-absence selection process calibrated to SDM performance by considering presence data. Other studies have found that randomly selected pseudo-absences yield the most reliable models (Barbet-Massin et al. 2012). The models were fit by assigning a number of pseudo-absences weighted to presences (Barbet-Massin et al. 2012), with an equal number of presences and absences.

Each type of SDM methodology has particular strengths and limitations in the way it accommodates the responses to predictors, as well as how it deals with missing observations. For example, linear regression fits linear functions relating a response variable to one or more predictor variables, where this relationship can be approximated by a straight line (Ferrier et al. 2002), whereas machine learning offers more complex classification algorithms that accommodate non-linear variable interactions (Salas et al. 2017). All algorithms were tested with replicated sub-sampling of 20% of the occurrence dataset.

Model evaluation methods

The contribution of SDMs in understanding the geographical distribution and abundance of a species depends on the level of reliability offered by the model (Barry and Elith 2006). The prediction accuracy must be assessed to determine the model’s suitability (Liu et al. 2009). Models can be judged on their capacity to discriminate presence from absence, which is measured by the number of false positive and false negative predictions. Several statistical indicators can be used as metrics to evaluate model performance (Fielding and Bell 1997). To assess the results here, the area under the Receiver Operating Characteristic (ROC) curve (AUC) value was used, as well as the correlation coefficient (COR) and the True Skill Statistics (TSS) value (Bradley 1997). The AUC value provides a single measure of model performance, showing the model’s ability to rank a randomly chosen presence observation higher than a randomly chosen absence observation (Liu et al. 2009). These values can range between 0 and 1; and models producing AUC values of 0.75 are regarded as reliable, 0.8 as good, and 0.9 to 1 as having excellent discriminating ability (Franklin 2009). The TSS is presented as an improved measure of model accuracy, defining the average of the net prediction success rates for presence sites and for absence sites (Allouche et al. 2006). The COR value allows another performance index comparison between models, and helps to validate the results obtained by the AUC and TSS methods (Elith et al. 2006). This study also ran null models, which make predictions in the absence of a particular ecological mechanism (Harvey et al. 2003), to assess the random probability hypothesis. The AUC values from the null models ranged from 0.51 to 0.53, thus corresponding to what would be expected by chance (Raes and ter Steege 2007). The distribution generated with a null model significantly differed from the other modelling results, with a predicted presence spread smoothly over most of the study area (Fig. 2 in Supplementary Materials).

As well as evaluating model performance with statistical indicators, the results were checked against the validation dataset (Anderson et al. 2003), consisting of the 25% of available presence data set aside for validation. The data distribution showed that 76% of the observation records within the presence range correspond to a threshold value of 0.9 to 1, and 86% were above a threshold of 0.8 (Table 5). Of the 690 occurrence locations set aside to check the results, 92% fell within the area predicted by the modelled map, in line with a presence probability threshold of 0.70.

Predictive maps generated by SDMs provide the occurrence probability of the species on a 0 to 1 scale. Threshold determination is a key step in transforming indices of suitability to binary predictions of species presence or absence (Nenzen and Araújo 2011). Threshold definition can be subjective or objective (Manel et al.

Table 5. Correspondence between observations and presence probability for the validation dataset. Values above a threshold of 0.7 are shown in bold.

Probability threshold	Number of observations	%
0.9 to 1	2,219	80.4
0.8 to 0.9	266	9.7
0.7 to 0.8	114	4.1
0.6 to 0.7	80	2.9
0.5 to 0.6	43	1.5
0.4 to 0.5	13	0.5
0 to 0.4	22	0.8

1999), and in many methods, the point at which sensitivity (true positive rate) and specificity (true negative rate) are equal can be chosen to determine the threshold. In this case, a value of 0.7 was chosen.

Results

All SDMs for the differing sets of variables performed well, demonstrating high predictive power (Table 6). The overall mean AUC value was 0.86, the mean TSS was 0.67, and the mean COR was 0.61. These results confirmed the hypothesis that SDMs based on bioclimatic variables could provide valuable results for the Ocel-lated Lizard. Moreover, the agreement between results validates the overall modelling methodology, including the quality of the sampling dataset and the geographical extent of the study. Overall, the initial trial made with Model 1 showed that it was a satisfactory model, with an AUC value of 0.91, a TSS value of 0.72, and a COR value of 0.78. The subsequent iterative trials carried out

with different combinations of variables further increased model accuracy, validating the most useful variables and helping to rank their contributions.

A comparison of the overall results led to the selection of Model 6 generated with the RF method as the most accurate model. The predictors of this model, based on selected bioclimatic variables with additional topographic and vegetation parameters, achieved the best performance using virtually all modelling methods, with maximum performance obtained from the RF method. This combination of variables and modelling method resulted in an AUC value of 0.98, a TSS value of 0.85, and a COR value of 0.88. An analysis of variance (ANOVA) was performed to confirm the validity of the chosen combination of variables. ANOVA results on Model 6 showed the lowest *p*-values for all variables, with a minimum value of 2.74e-14 and a maximum value of 0.020393 (Table 7). In contrast, models 1 to 5 each included a variable with a *p*-value > 0.05. As a study by Wood et al. (2016) mentioned that the Akaike information criterion (AIC) works reasonably well for model selection, the AIC between models were also compared here, and this comparison showed that Model 6 had the lowest AIC values (Table 2 in Supplementary Materials).

Contribution of Variables

The contributions of each variable in Model 6 were ranked, identifying four critical parameters, as well as one secondary factor (Table 8). The four main factors that most influenced model performance were (according to their relative importance): precipitation in the driest month, temperature seasonality, mean temperature in the driest quarter, and NDVI. Precipitation seasonality was

Table 6. Comparison of model results based on different modelling methods and assessments of model performance. The values in bold, for Model 6 and RF, indicate the results with the highest accuracy.

Model	BRT			CART			GAM			GLM		
	AUC	COR	TSS	AUC	COR	TSS	AUC	COR	TSS	AUC	COR	TSS
M1	0.82	0.59	0.55	0.87	0.74	0.65	0.87	0.74	0.66	0.83	0.59	0.48
M2	0.85	0.63	0.62	0.84	0.73	0.66	0.86	0.73	0.67	0.83	0.63	0.56
M3	0.85	0.6	0.57	0.82	0.65	0.54	0.88	0.71	0.69	0.83	0.57	0.58
M4	0.8	0.55	0.52	0.82	0.71	0.65	0.9	0.73	0.63	0.82	0.59	0.5
M5	0.82	0.54	0.52	0.83	0.67	0.61	0.8	0.75	0.7	0.83	0.57	0.5
M6	0.82	0.56	0.48	0.85	0.66	0.62	0.91	0.67	0.6	0.84	0.57	0.52

Model	MARS			MAXLIKE			RF			SVM		
	AUC	COR	TSS	AUC	COR	TSS	AUC	COR	TSS	AUC	COR	TSS
M1	0.86	0.72	0.66	0.82	0.59	0.48	0.91	0.78	0.72	0.84	0.71	0.65
M2	0.84	0.68	0.64	0.83	0.62	0.52	0.92	0.8	0.75	0.89	0.75	0.74
M3	0.89	0.71	0.66	0.82	0.54	0.52	0.93	0.78	0.73	0.88	0.75	0.71
M4	0.88	0.69	0.65	0.79	0.55	0.48	0.94	0.79	0.77	0.88	0.74	0.71
M5	0.9	0.74	0.67	0.83	0.56	0.49	0.95	0.8	0.76	0.89	0.73	0.72
M6	0.88	0.65	0.59	0.82	0.56	0.48	0.98	0.87	0.85	0.9	0.7	0.67

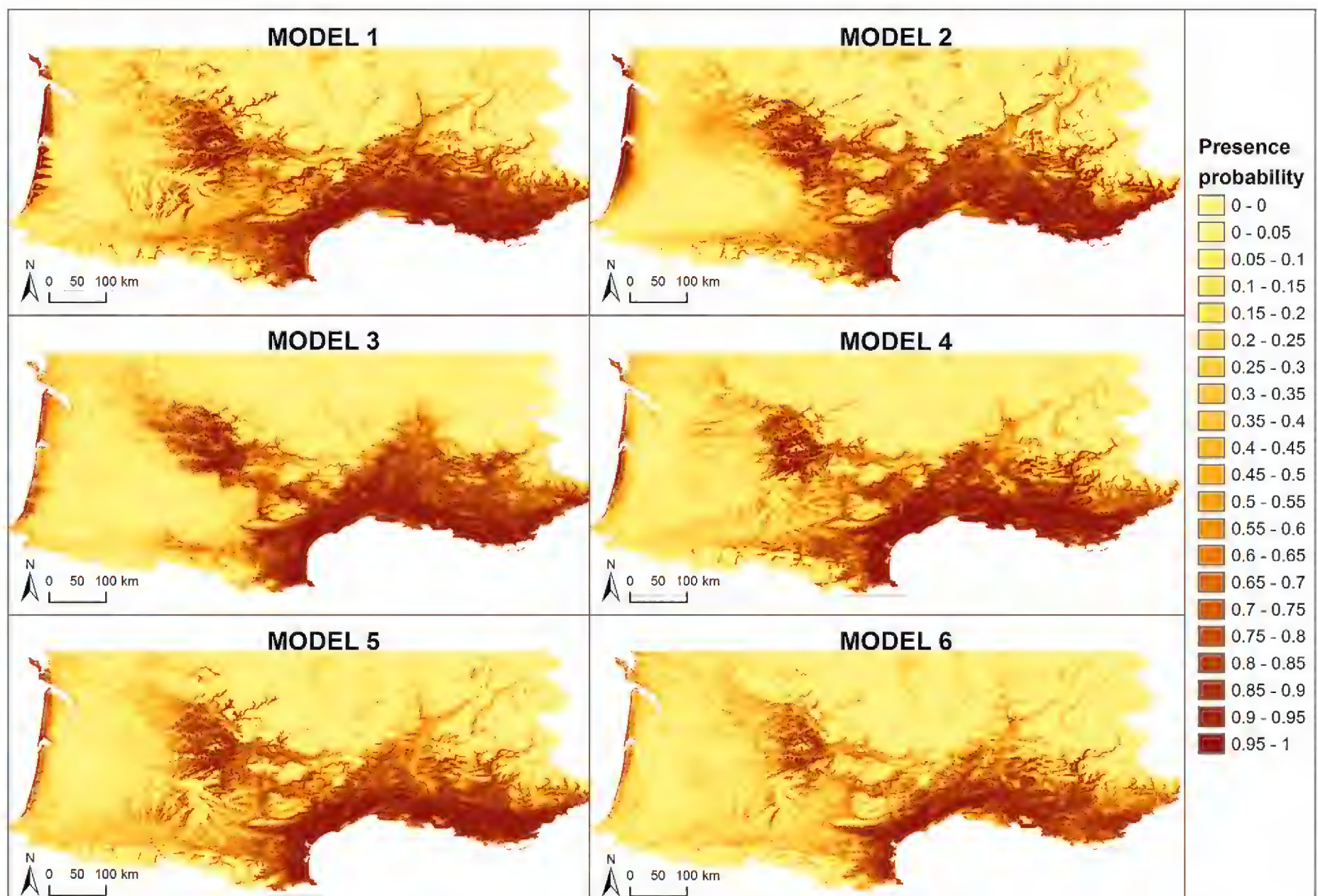


Fig. 6. Predictive modelling maps showing presence probability of the Ocellated Lizard (*Timon lepidus*) in the study area.

also a contributing variable, but only to a minor extent. Climatic factors associated with dry and warm weather conditions seem to play a determining role in the spatial distribution of the Ocellated Lizard, with precipitation in the driest month being the primary contributing factor. The contributions of these variables can be interpreted as a reflection of the species' ecological needs, especially as related to its reproductive cycle.

Predictive Habitat Suitability Maps

The predictive maps (Fig. 6) show very slight differences between the six models. Oléron Island, where the most northerly currently known population of this species is found, is included in all models, but with different probability ranges. Its presence is predicted on the whole island in models 1, 2, 4, and 5, but only on part of the island in models 3 and 6. All models show a clear link between the 'Mediterranean population' and the 'Lot population' (in a region lying northwest of the Mediterranean), with minor variations in the continuity of the species distribution between these two 'populations.' In the different maps, the penetration of the species into the Rhône Valley appears more or less extended, and the fragmentation of the 'Lot population' is more or less pronounced. Apart from these details, the maps based on the six models are extremely consistent.

On the map generated by Model 6 (Fig. 7), most of the locations historically occupied by the species (i.e., for which evidence exists of its disappearance) are along the Atlantic coast (in the Nouvelle-Aquitaine region), the area where the distribution of the species is the most limited and fragmented (Fig. 8). The disappearances of the three Mediterranean populations correspond to very specific cases: two islands (Ratonneau and Porquerolles islands), where the species is likely to have disappeared as a result of the introduction of predators (Cheylan 2016), and a population in the Rhône delta in the Camargue, where the decline of rabbits has transformed the environment, leading to the disappearance of the Ocellated Lizard in this area (Doré et al. 2015).

Identification of Knowledge Gaps

The potential distribution predicted by Model 6 indicates that knowledge gaps regarding the observed presence of this species are considerable, not only in the Mediterranean distribution range, but also in its periphery (Fig. 9). Allowing a buffer zone with a 5-km radius around each observation location, only 74% of the area of predicted presence (based on Model 6 with a presence probability threshold of 0.70) is confirmed by actual observations; observation data is lacking for 26% of the area. In the core of the distribution range,

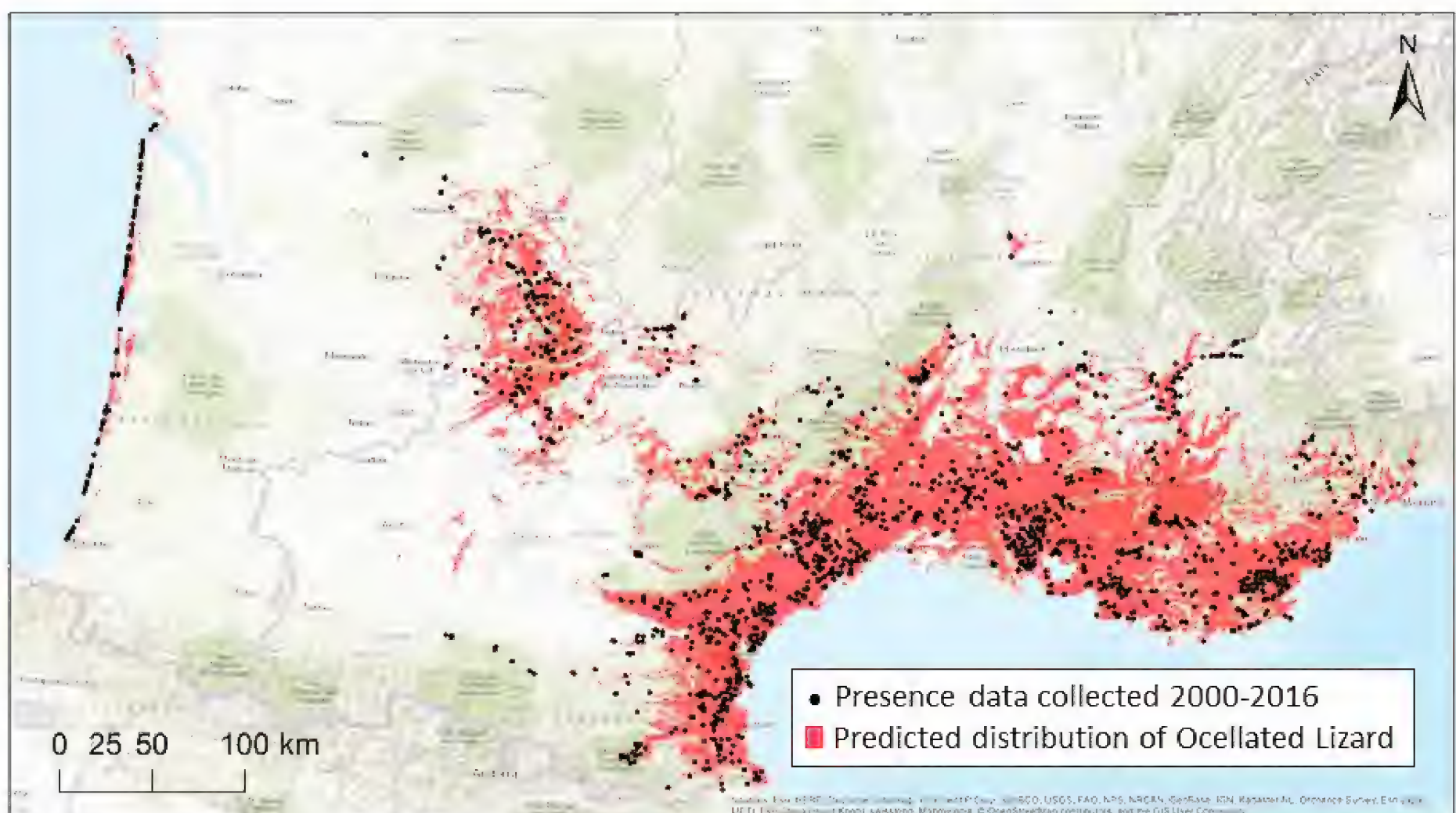


Fig. 7. The predicted distribution map generated by Model 6 (in pink) with specific locations of presence data (from observations) from the dataset (black dots).

information is missing in several areas of the regions of Provence and in a few areas of Languedoc-Roussillon. This is particularly the case for several noteworthy zones.

- Var department (e.g., around Fayence, Draguignan, Bargemon, Seillons-source-d'Argens, and Saint-Maximin-la-Sainte-Baume)
- Southeast of Alpes-de-Haute-Provence (e.g., the Valensole plateau, the Asse-Puimichel valley, and the lower Bléone valley)
- Southeast and northwest of the Vaucluse department (e.g., Pertuis, La Tour-d'Aigues, Carpentras, Uchaux, Sainte-Cécile-les-Vignes, and Valréas)
- Drôme department (Valence plain, Montélimar plain, Tricastin, and Baronnies Provençales)
- Gard department (e.g., Saint-Quentin-la-Poterie, Bagnols-sur-Cèze, Alès, and La-Grand-Combe)
- Southeast of Ardèche (e.g., Saint-Thomé, Villeeneuve-de-Berg, and Saint-Marcel-d'Ardèche)
- South and far northwest of the Lozère (e.g., Aysséries, Faveyrolles, La-Bastide-Solages, Sébrassac, Decazeville, and Claunhac)

In western Languedoc-Roussillon, it would seem relevant to look for the Ocellated Lizard in several zones of the Aude department (e.g., Carcassonne, Labécède-Lauragais, Rouffiac-des-Corbières, and Palairac), and in several areas of the Pyrénées-Orientales (e.g., Saint-Paul-de-Fenouillet, and La Trinité) and the

Hérault (e.g., Pézenas and Aigues-Vives). Outside of the Mediterranean region, areas where this species would merit further survey efforts are more numerous, notably in the Aveyron, Tarn, Haute-Garonne, Tarn-et-Garonne, Lot, and in Dordogne, where the potential distribution area is very fragmented and the natural habitats small (Berroneau 2012; Pottier et al. 2017). To address this, surveys could be carried out in several areas of the Tarn and the Lot (e.g., Mazamet, Roquecourbe, Larroque, Lacapelle-Marival, Prayssac, Gourdon, and Martel), in southwest Corrèze (e.g., Tulle, Taurisson, and Saint-Aulaire), in southeast Dordogne (e.g., Carsac-Aillac, Hautefort, and Rouffignac-Saint-Cernin-de-Reilhac), and in eastern Tarn-et-Garonne (e.g., Caylus). All of these areas possess a high presence probability of this species according to the model results, so it is likely that inadequate surveying ex-

Table 7. ANOVA produced by the Generalized Additive Model for Model 6. Approximate significance of smooth terms.

	Edf	Ref.df	Chi.sq	p-value
s(bio4_R)	7.928	8.263	84.87	2.74e-14
s(bio9_R)	7.445	7.853	38.47	5.88e-06
s(bio10_R)	6.469	6.779	42.75	7.37e-07
s(bio14_R)	7.608	8.248	18.61	0.020393
s(bio15_R)	6.071	7.297	18.72	0.008308
s(bio16_R)	7.944	8.688	18.79	0.018410
s(alt250_R)	6.279	6.819	34.27	9.87e-06
s(orient250_R)	3.392	4.241	20.47	0.000507
s(median_MO-DIS2)	2.734	3.462	40.17	3.36e-08

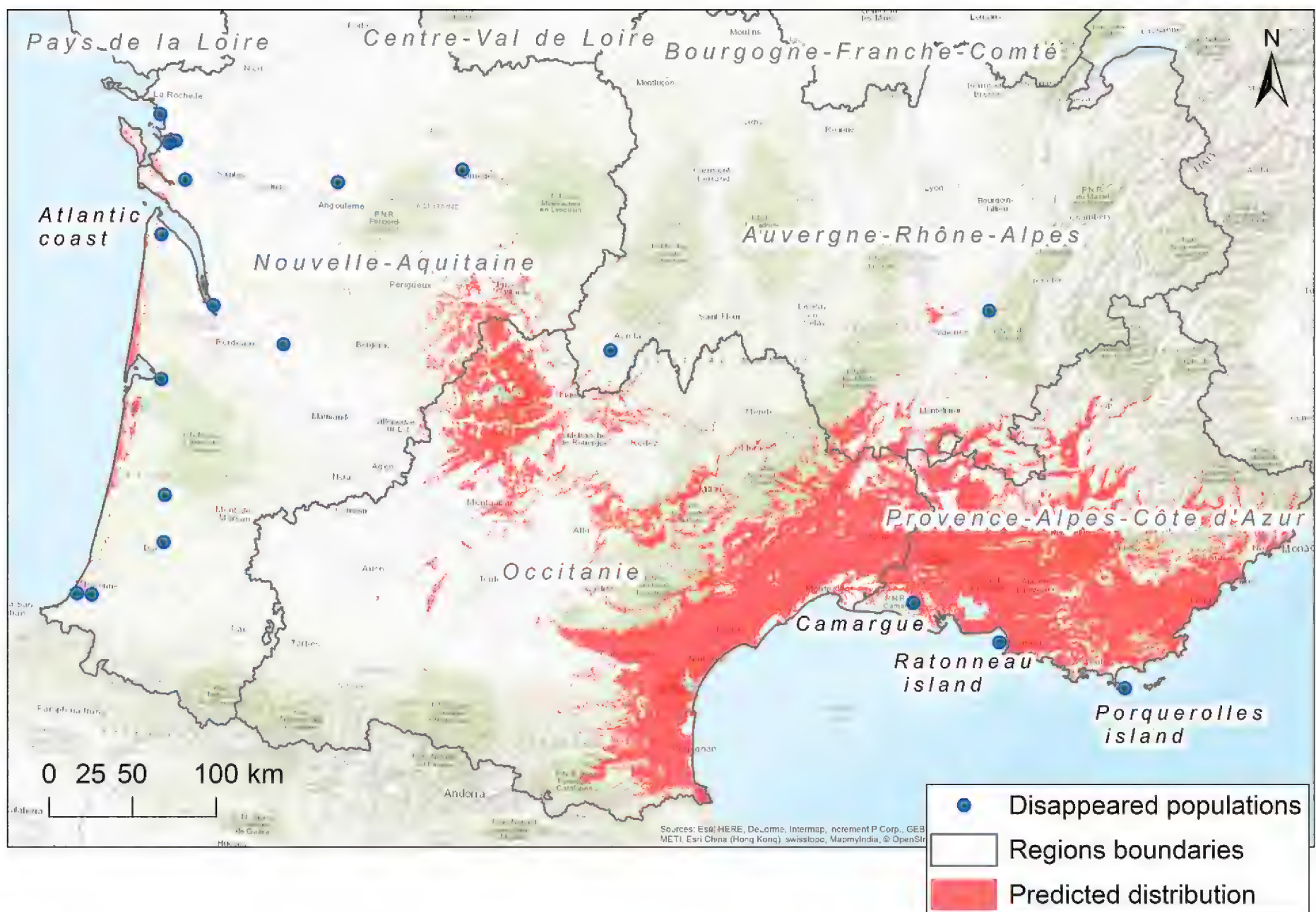


Fig. 8. Location of populations that have disappeared in relation to the predicted presence map generated by Model 6 (blue dots).

plains the current absence of data in these locations.

Contribution of Protected Areas to the Conservation of the Species

A comparison of the potential niche (Model 6 with a presence probability of ≥ 0.70) and the main national protected areas allowed an assessment of the contribution of the current network of natural reserves to the conservation of the species (Table 9).

The map in Fig. 10 shows the protected areas that contribute most to the conservation of the species are the regional nature parks (representing 22% of its predicted niche) and the two Natura 2000 zones (15% and 16%). The other types of protected areas contribute to a much more limited extent, covering between only 0.1% and 2% of the potential distribution area of 35,805 km². As these protected areas often overlap (regional nature parks often include nature reserves, and are generally also part of the Natura 2000 network), it is difficult to calculate the surface areas to evaluate the total contribution of all the protected sites. It should be noted that the protected areas with the strongest regulatory protection (national parks, national and regional nature reserves, and National Forest Agency ecological reserves) cover less than 1% of the potential niche of this species.

Discussion

Model Performance

Nine variables, out of the selection of 28 climate variables, three topographic variables, and three vegetation variables, were found through iteration to contribute most effectively to the quality of the models. The tests of six models with eight statistical algorithms led to very sound results, confirming the performance of the models. The consistency between the models and the statistical soundness of the modelling are largely due to the good spatial coverage of the source data, its geographical precision, and the size of the dataset (Araújo and Guisan 2006).

Given the exhaustive nature of the occurrence data, we feel confident that the predictive model of the ecological niche gives a fairly good picture of the potential distribution of the species, and can help to map its actual current distribution. Other studies have demonstrated that SDMs can accurately determine the natural distribution of a species, contributing to the more complete knowledge of its current range (Elith and Leathwick 2009).

In this study, the RF method resulted in the most accurate SDMs, performing well with all the sets of variables. This method uses decision trees based on random grouping of the covariates, modelling both the interactions be-

Table 8. Variable importance index generated by the best SDM, sorted by rank of importance.

Variable	Description	Cor_Test	AUC_test	Rank
bio14	Precipitation in the driest month	0.1794	0.0330	1
bio4	Temperature seasonality (std*100)	0.1172	0.0216	2
bio9	Mean temperature in the driest quarter	0.1012	0.0168	3
ndvi	Normalized Vegetation Index	0.0801	0.0152	4
bio15	Precipitation seasonality (coefficient of variation)	0.0666	0.0097	5
bio10	Mean temperature in the warmest quarter	0.0335	0.0060	6
bio16	Precipitation in the wettest quarter	0.0394	0.0018	7
altitude	Elevation	0.0575	0.0012	8
aspect	Slope orientation	0.0219	0.0004	9

tween the variables and their nonlinear relationships, and it uses bootstrapping to fit individual trees (Salas et al. 2017). Cutler (2007) demonstrated that the advantages of RF include very high classification accuracy, ability to model complex interactions between predictor variables, and an algorithm for imputing missing values. This gives RF the flexibility to perform several types of statistical data analysis, including regression, classification, survival analysis, and unsupervised learning (Cutler 2007). Rangel and Loyola (2012) also demonstrated that machine learning methods such as RF have high statistical precision and predictive power for determining the species distribution of well-known populations.

While the models here showed high accuracy, certain improvements could be made by integrating additional

datasets not included in this study, such as soil maps, the distribution of tree species, or detailed vegetation maps of France (Legu dois et al. 2011). While land cover parameters could contribute to identifying habitat suitability, their integration in SDMs remains a challenge in terms of accuracy and validity (e.g., in terms of how current they are). The challenges of incorporating land cover data are due to the fact that they are categorical variables with limitations imposed by spatial resolution, date of production, and thematic classification. Studies have shown that continuous remotely sensed predictor variables offer many advantages over categorical variables and can be used effectively in species distribution modelling (Wilson et al. 2013). Models based on bioclimatic variables have proven their efficiency in numerous

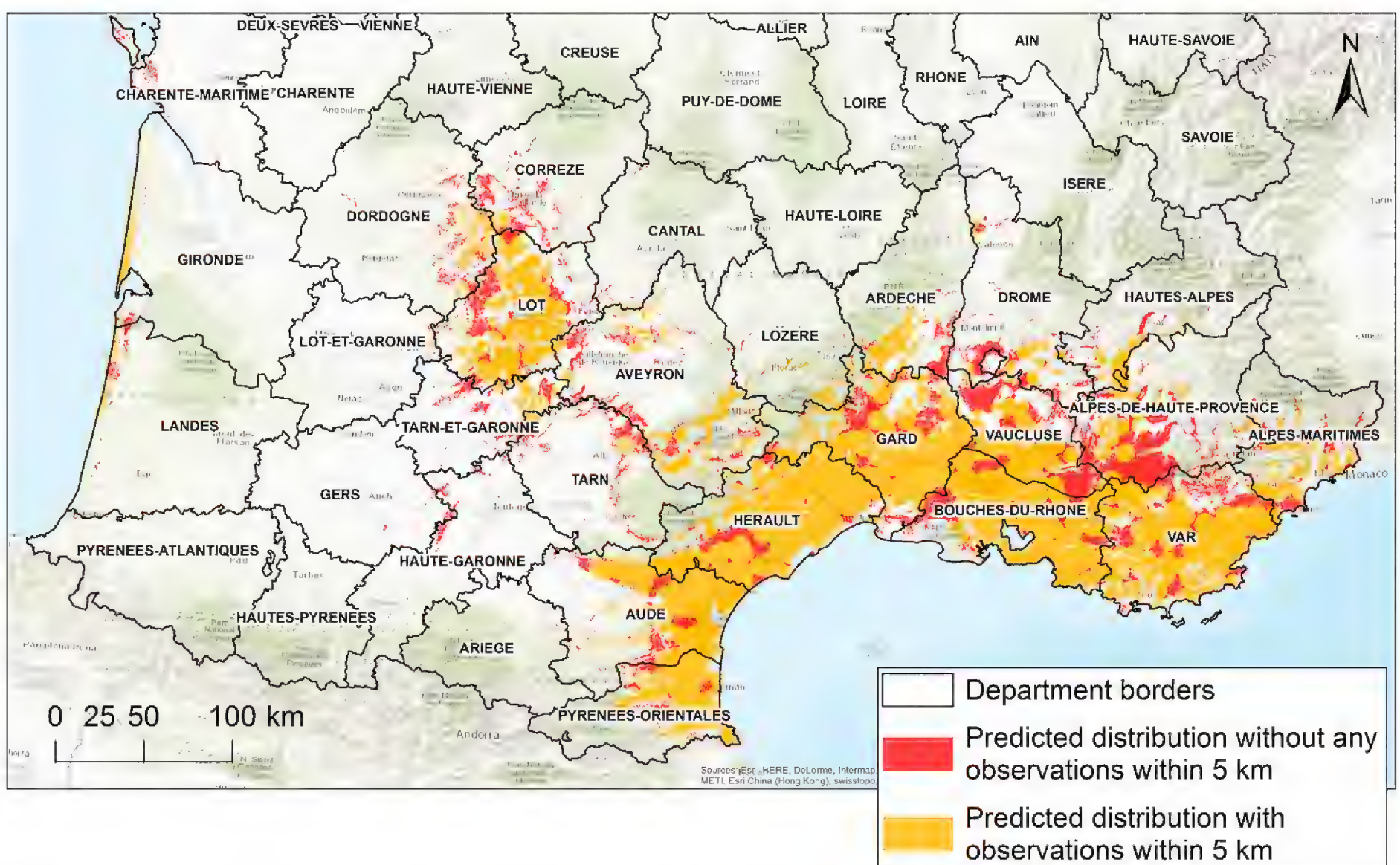


Fig. 9. Areas within the potential niche of the Ocellated Lizard (in orange) for which there are no observation data (in red), based on Model 6 with its presence probability threshold of 0.70 combined with presence data from observations including a buffer zone with a 5-km radius.

Table 9. The contributions of the different types of protected areas to the conservation of the Ocellated Lizard in France. The surface area favorable to the presence of the species (“potential niche” in header of second column) is given in km² according to Model M6. The percentage of the protected area relative to the total surface area of the predicted distribution range is shown on the third column.

Type of protected area	Surface area under protection within the total potential niche (km ²)	Percentage of the potential niche*
Regional Nature Park	8,121	22.68
Special Area of Conservation (Natura 2000)	5,927	16.55
Special Protection Areas (Natura 2000)	5,585	15.60
National Park (park peripheral zone)	792	2.21
National Nature Reserve	170	0.47
National Park (park core area)	92	0.26
Ecological Reserve (National Forest Agency)	43	0.12
Regional Nature Reserve	20	0.06

* Total surface area predicted with a probability threshold of 0.7 = 35,805 km².

studies, while one study has shown that the addition of land cover variables to pure bioclimatic models does not necessarily improve the predictive accuracy of the resulting SDM (Thuiller et al. 2004).

Consistency between Potential and Realized Distributions

The results obtained here showed strong agreement between the predictive models and the observed distribution of the Ocellated Lizard on a macro-geographic scale, giving rise to several conclusions. The first is that climatic predictors prevail over all the other predictors for this species. The current boundaries of the distribution range

of the Ocellated Lizard in France are essentially defined by climatic factors, which aligns with many studies on reptiles (Guisan and Hofer 2003; Santos et al. 2009; Brito et al. 2011). This suggests an ancient presence of the species in France, given the barriers to dispersion such as rivers and mountains in its territory, and the time necessary to colonize the entire potential bioclimatic niche. The fragmentation of the populations at the edges of the distribution, as well as the historical information regarding the loss of populations (Cheylan and Grillet 2005; Doré et al. 2015), support this idea and suggest the existence in the past of a larger and, above all, a less fragmented range. Unfortunately, zooarchaeological information on this subject is limited. The species is known to have been

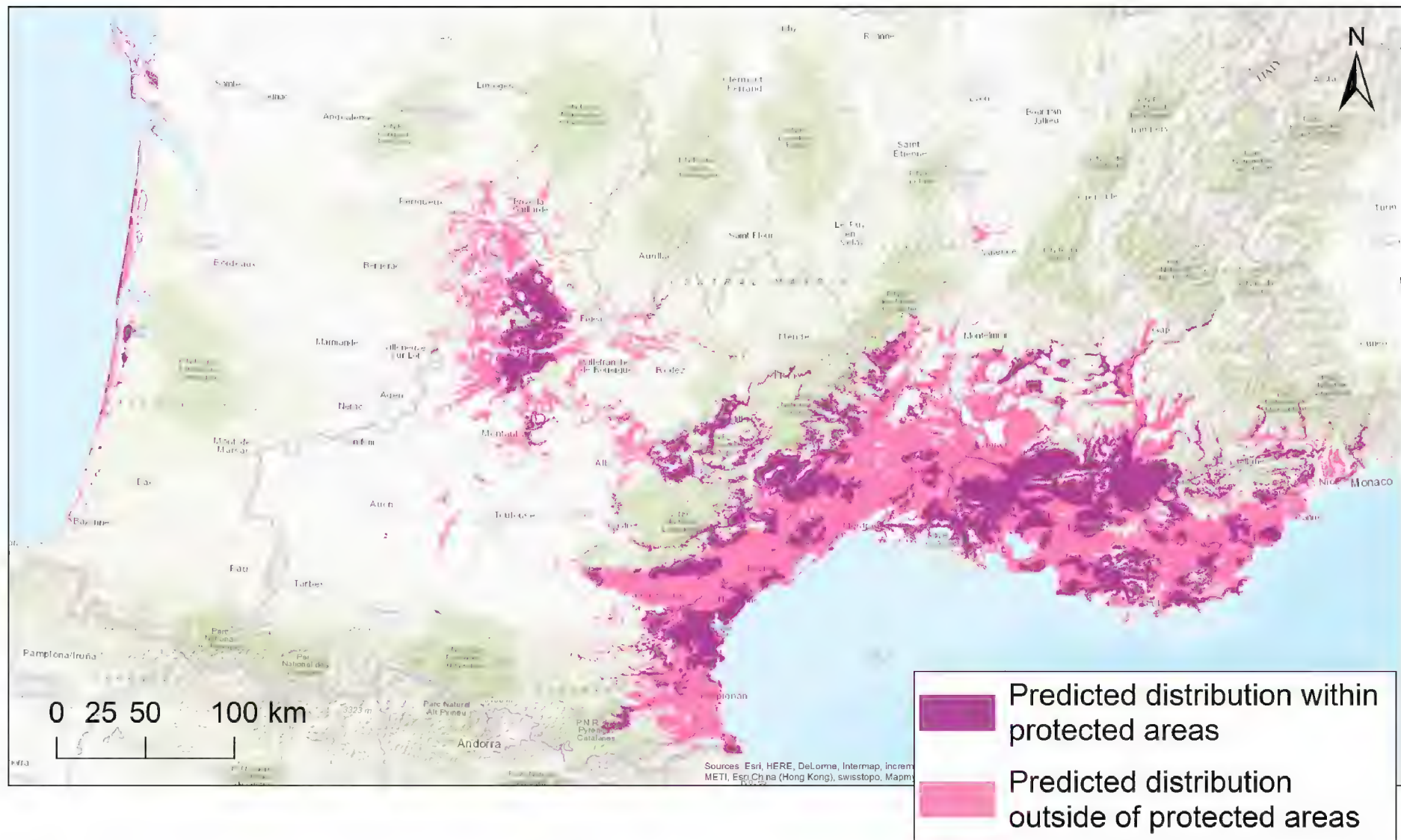


Figure 10: Contribution of protected nature areas to the conservation of *Timon lepidus*. Predicted presence within protected areas (dark purple) and predicted distribution range (light purple).

present in France in the Middle Pleistocene (~700,000 to 150,000 yr ago), from remains in the Lazaret cave in Nice (Bailon 2012), and remains from the Holocene have also been found (Mateo 2011). However, the lack of fossil remains from the last interglacial optimum (between 125,000 and 11,000 yr ago) does not definitively prove the retreat of the species to the Iberian Peninsula during this period. The presence of isolated populations on the northern edges of the current distribution, as well as its presence in Liguria, would have required overcoming major obstacles (the Rhône, Var, and La Roya rivers), which points to an ancient occupation of the territory. Given these factors, the hypothesis that the species remained during the interglacial period seems possible, at least in the far south of France.

The strong concurrence between the models and the observed distribution also indicates that the process of decline in this species is moderate, as all the areas favorable to the species are still occupied, apart from a few exceptions. Even at a lower spatial resolution, the boundaries of the distribution range are primarily due to climatic factors, and only secondarily to ecological factors (i.e., presence of favorable habitat). This is particularly true at the edges of its distribution range in the valleys that open onto the Mediterranean coast (i.e., those of the Aude, Rhône, Durance, and Var), where the extent of the penetration of the species coincides with the boundary of the Mediterranean climate and vegetation, as there are no physical obstacles preventing a deeper advance in these valleys (Deso et al. 2011, 2015). Notably, the model clearly differentiates areas favorable to the Ocellated Lizard in zones of rugged terrain. This is particularly the case in the region of the Causses (in the southern part of Aveyron), which is characterized by limestone plateaus that would potentially be favorable to the species but where it is not present, and by deep gorges (the valleys of the Tarn and the Jonte) where the species has long been observed. Rather surprisingly, the model distinguished between these two zones (plateaus and gorges) despite any notable climatic difference between them.

On the other hand, several areas not predicted by the model have the proven presence of the species (e.g., the foothills of the Pyrénées in Ariège, the mountainous zones of Ardèche, and northern Dordogne). This is likely explained by the resolution of the model, which is ill-adapted to predicting very small areas within a landscape and climate matrix that is generally unfavorable to the species. These known populations live in very small micro-habitats (a few dozen ha at most) with unique botanical characteristics distinct from the surrounding landscapes. An analysis that takes into account a finer landscape scale, particularly in terms of vegetation, would produce a model with a closer fit. Equally, substrate characteristics, which were not taken into account in the model, play an important role in the presence of the Ocellated Lizard when the climatic environment is unfavorable. In this case, it seeks out terrain that is rather

steep, rocky, or well drained to avoid environments that are too wet.

Which Variables Best Explain the Distribution of the Species?

At the macro-geographic scale, the variables that best explain the distribution of the species are related to climate and, to a lesser extent, vegetation and topography. This indicates the primacy, over all other variables, of a hot and dry summer, as well as a strong seasonal contrast; two key characteristics of the Mediterranean climate (Blondel et al. 2010). The importance of temperature and aridity in the summer is certainly due to the reproduction requirements of this species. In France, female Ocellated Lizards are known to typically lay their eggs at the end of May or the beginning of June (Cheylan and Grillet 2004), and the eggs hatch the third week of September or the first week of October (Bischoff et al. 1984; Doré et al. 2015). In Provence, this corresponds to an incubation period of about 100 days (Cheylan and Grillet 2004). Hence, the entire summer period is used for reproduction. The late hatching period requires mild temperatures at the end of summer and beginning of autumn, allowing the hatchlings to feed before the hibernation period, which begins around 15 November in most of the French regions where this species is present (Doré et al. 2015).

At the local scale, the presence of the species is primarily influenced by the aridity of the habitat; the Ocellated Lizard prefers a rocky or well-drained substrate that is well exposed to the sun. Dense vegetation cover is very unfavorable for this species, as shown in a study by Santos and Cheylan (2013) in Provence. In the future, gaining a better understanding of the importance of each of these habitat variables would be useful, drawing upon the resources available on the subject.

Why is this Species Retreating at the Edges of its Distribution Range, in Contrast to Climatic Expectations?

The proven extinction of several Ocellated Lizard populations over the last 150 years, mainly on the northern border of its distribution range (Cheylan and Grillet 2005; Grillet et al. 2006) runs counter to what might be expected with the warming of the climate, the effects of which have been clearly demonstrated on Mediterranean reptiles in the south of France (Prodon et al. 2017). Given its high thermal requirements, this species should in fact benefit from climatic warming, particularly at the northern edge of its distribution. However, the opposite is observed, which suggests the predominance of local over global factors. Studies carried out to investigate this issue have shown that several local factors explain the decline (or even the disappearance) of local populations of this species. Those factors include the introduction of predators in the case of island populations (Cheylan

2016), the disappearance of the European rabbit and the resulting changes to the landscape (Grillet et al. 2010), the impact of pest control on entomofauna prey (Doré et al. 2015), and the abandonment of agricultural land and the resulting progression of woodland (Grillet et al. 2006; Pottier et al. 2017). Thus, the expected effects on reptiles of the changes caused by warming in Europe (Araújo et al. 2006) are not borne out in the case of the Ocellated Lizard.

Which Zones should be Surveyed in the Future to Improve Our Knowledge of this Species' Distribution?

The recent discovery of a population in Vendée (Cédric Baudran, pers. comm. 2018), beyond the known boundary on the Atlantic coast, shows that new populations remain to be discovered, especially at the edges of the distribution range. A priority would be to seek confirmation of the true disappearance of the species in selected sites where it was known in the past, based on the presence predictions generated by the SDMs. Secondly, an attempt to confirm the existence of connections between population clusters that are considered to be separated would be interesting. This would be particularly useful for populations located in the mountainous zones of the Alpes-Maritimes (Deso et al. 2015), in the upper Durance valley (Deso et al. 2011), and in the Rhône valley (Doré et al. 2015), as well as the fragmented populations in the Lozère, Aveyron, Tarn, Tarn-et-Garonne, Lot, Dordogne, Corrèze, and Cantal (Geniez and Cheylan 2012; Pottier et al. 2017). The coastal populations of the Atlantic currently seem rather well-defined (Berroneau 2012); however, this does not exclude the possibility of discovering new populations there.

What Conservation Strategy should be Adopted to Protect this Species?

The predictive distribution models generated in this study provide interesting leads for defining a conservation strategy for this species. First, the current network of protected areas in France can be considered to rather satisfactorily cover the distribution range of the Ocellated Lizard and its different population clusters. However, a deeper analysis reveals that only a very small proportion of the area potentially favorable to the species benefits from strong protection regulations. The areas of land with the strictest protection (national and regional nature reserves, National Forest Agency ecological reserves, and national parks) only represent 1.2% of the potential niche of this species in France. In terms of national parks, the Cévennes National Park clearly bears the most responsibility in terms of the conservation of this species, followed at some distance by the Calanques National Park (respectively, 40 and 95 km² of favorable habitats for the species). There are 15 national nature reserves with the presence of the species, and in this category of

protected area, the reserves of Coussouls de Crau and the Maures plain have the largest known populations (respectively, 74 km² and 52 km² of favorable habitat). Of National Forest Agency ecological reserves, 16 include land where the lizard is found, with the largest being the Maures reserve (18 km² of favorable habitat) and the Petit Luberon reserve (16 km² of favorable habitat). Some 240 Natura 2000 sites have conditions that are potentially favorable to the species; 21 protect areas of land that contribute to the conservation of the species of more than 90 km², for a total contribution of about 3,430 km² for this category of protected area.

Given the rather dense network of protected areas, both in terms of spatial extent and altitudinal range, a strategy based on anticipating climate change (Salas et al. 2017) is not necessarily the best choice. As stated above, this species is in decline at the northern edge of its distribution, which runs counter to the expected effects of a warming climate (which is predicted for the region in the future). Moreover, the refuge habitat for this species (and where it originated) is located in the southern half of the Iberian Peninsula (Miraldo et al. 2011), so the effects of climatic warming are unlikely to be harmful to the species at the northern edge of its distribution. In support of this hypothesis, it has been argued that the increase in wildfires due to climatic warming will significantly increase the density of Ocellated Lizard populations in the Mediterranean region, by transforming woodland into open landscapes (Santos and Cheylan 2013). A more important consideration than climatic warming for the conservation of the species is that its spread relies on the existence (or not) of favorable environments, and its demographic capacity to colonize new territories. Unfortunately, studies of the isolated populations at the edges of the distribution range (Grillet et al. 2006; Deso et al. 2015; Pottier et al. 2017) show that these two parameters are rarely present, and that these populations are, in the more or less long term, undergoing a process of extinction (Salvidio et al. 2004; Cheylan and Grillet 2005).

From a strategic point of view, therefore, the core of the distribution range should be prioritized for conservation efforts in the long term, without neglecting certain peripheral populations in the shorter term (e.g., the populations in the valleys of the Durance, Rhône, and Var rivers, and the sandy habitats of the Atlantic coast). Our SDM-generated maps indicate that the isolated populations of the Atlantic coast, as well as the population clusters west of the Massif Central (in the departments of Aveyron, Tarn, Tarn-et-Garonne, Lot, and Dordogne), offer climatic and topographical conditions that are very favorable to this species. These populations, while isolated from the Mediterranean population, should be given careful attention. They may even harbor specific genetic compositions that warrant further consideration.

The strong dependence of the Ocellated Lizard on the European rabbit in soft soils (Grillet et al. 2010) also suggests the value of taking concerted conservation mea-

tures that equally protect this mammal. As the demographic trends of rabbit populations in the Mediterranean region are very negative (Ward 2005; Delibes-Mateos et al. 2008; Poitevin et al. 2010), this could result in a domino effect on Ocellated Lizard populations.

Conclusions

This analysis, carried out at the scale of France, reveals that the distribution of the Ocellated Lizard is primarily conditional on climatic factors, in particular the length of the arid summer period. Further study at a smaller scale would help to provide a more detailed understanding of the ecological preferences of this species. Such a study could consider two finer, overlapping spatial scales: the Mediterranean coast and the region around Montpellier. Focusing on the Mediterranean coastal plains would allow climatic and topographic variables to be separated out, at least partially, to better bring to light the roles of factors linked to land use. Including the region of Montpellier would more completely isolate climatic and topographic variables, allowing a focus on habitat variables directly linked to the ecology of the species: soil type, crop or natural vegetation type, level of urbanization, and the presence and density of European rabbits.

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Literature Cited

- Allouche O, Tsoar A, Kadmon R. 2006. Assessing the accuracy of species distribution models: prevalence, kappa, and the true skill statistic (TSS). *Journal of Applied Ecology* 43: 1,223–1,232.
- Anderson RP, Martinez-Meyer H. 2004. Modeling species’ geographic distributions for preliminary conservation assessments: an implementation with the spiny pocket mice (*Heteromys*) of Ecuador. *Biological Conservation* 116: 167–179.
- Anderson RP, Lew D, Peterson AT. 2003. Evaluating predictive models of species’ distributions: criteria for selecting optimal models. *Ecological Modelling* 162: 211–232.
- Araújo MB, Segurado P. 2004. An evaluation of methods for modelling species distributions. *Journal of Biogeography* 31: 1,555–1,568.
- Araújo MB, Guisan A. 2006. Five (or so) challenges for species distribution modelling. *Journal of Biogeography* 33: 1,677–1,688.
- Araújo MB, Thuiller W, Pearson RG. 2006. Climate warming and the decline of amphibians and reptiles in Europe. *Journal of Biogeography* 33(10): 1,712–1,728.
- Austin M. 2002. Spatial prediction of species distribution: an interface between ecological theory and statistical modelling. *Ecological Modelling* 157: 101–118.
- Austin MP, Nicholls AO, Argules CR. 1990. Measurement of the realized qualitative niche: environmental niches of five *Eucalyptus* species. *Ecological Monographs* 60: 161–177.
- Bailón S. 2012. Données fossiles et mise en place de l’herpétofaune actuelle de la France. Pp. 33–39 In: *Atlas des Amphibiens et Reptiles de France*. Coordinators, Lescure J, Massary de J-C. Biotopie, Mèze, France and Muséum National d’Histoire Naturelle, Paris, France. 272 p.
- Barbet-Massin M, Jiguet F, Albert CH, Thuiller W. 2012. Selecting pseudo-absences for species distribution models: how, where, and how many? *Methods in Ecology and Evolution* 3(2): 327–338.
- Barbosa AM, Real R, Olivero J, Vargas JM. 2003. Otter (*Lutra lutra*) distribution modeling at two resolution scales suited to conservation planning in the Iberian Peninsula. *Biological Conservation* 114(3): 377–387.
- Barry S, Elith J. 2006. Error and uncertainty in habitat models. *Journal of Applied Ecology* 43(3): 413–423.
- Bashfield A, Keim A. 2011. Continent-wide DEM creation for the European Union. Pp. 10–15 In: *34th International Symposium on Remote Sensing of Environment. The GEOSS Era: Towards Operational Environmental Monitoring*. Sydney, Australia. CSIRO, Clayton, Victoria, Australia. 788 p.
- Berroneau M. 2012. *Guide Technique de Conservation du Léopard Ocellé en Aquitaine*. Association Cistude Nature, Le Haillan, France. 118 p.
- Bischoff W, Cheylan M, Böhme W. 1984. *Lacerta lepida* Daudin 1802 - Perleidechse. Pp. 181–210 In: *Handbuch der Reptilien und Amphibien Europas: II*. Editor, Böhme W. Akademische Verlagsgesellschaft, Wiesbaden, Germany. 416 p.
- Blondel J, Aronson J, Bodiou JY, Boeuf G. 2010. *The Mediterranean Region: Biological Diversity in Space and Time*. Oxford University Press, New York, New York, USA. 376 p.
- Bradley A. 1997. The use of the area under the ROC curve in the evaluation of machine learning algorithms. *Pattern Recognition* 30: 1,145–1,159.
- Breiman L. 2001. Random forests. *Machine Learning* 45(1): 5–32.
- Breiman L, Friedman J, Olsen R, Stone C. 1984. *Classification and Regression Trees*. Wadsworth and Brooks, Belmont, California, USA. 358 p.
- Brito JC, Brito-e-Abreu F, Paulo OS, Rosa HD, Crespo EG. 1996. Distribution of Schreiber's green lizard (*Lacerta schreiberi*) in Portugal: a predictive model. *Herpetological Journal* 6: 43–47.

- Brito JC, Fahd S, Martínez-Freiría F, Tarroso P, Larbes S, Pleguezuelos JM, Santos X. 2011. Climate change and peripheral populations: predictions for a relict Mediterranean viper. *Acta Herpetologica* 6(1): 105–118.
- Brotons L, Thuiller W, Araújo MB, Hirzel AH. 2004. Presence-absence versus presence-only modelling methods for predicting bird habitat suitability. *Ecography* 27: 437–448.
- Bucklin DN, Basille M, Benscoter AM, Brandt LA, Mazzotti FJ, Romanach SS, Watling JI. 2015. Comparing species distribution models constructed with different subsets of environmental predictors. *Diversity and Distributions* 21(1): 23–35.
- Carvalho SB, Brito JC, Crespo EG, Watts ME, Possingham HP. 2011. Conservation planning under climate change: Toward accounting for uncertainty in predicted species distributions to increase confidence in conservation investments in space and time. *Biological Conservation* 144(7): 2,020–2,030.
- Chebib A, Badeau V, Boe J, Chuine I, Delire C, Dufrene E, Francois C, Gritti ES, Legay M, Page C, et al. 2012. Climate change impacts on tree ranges: Model intercomparison facilitates understanding and quantification of uncertainty. *Ecology Letters* 15(6): 533–544.
- Cheylan M. 2016. The extinction of the Ocellated Lizard *Timon lepidus lepidus* (Daudin, 1802) on the island of Porquerolles (Provence, France). *Boletín de la Asociación Herpetológica Española* 27(2): 155–158.
- Cheylan M, Grillet P. 2004. *Le Léopard Ocellé*. Eveil Nature. Editions Belin, Paris, France. 95 p.
- Cheylan M, Grillet P. 2005. Statut passé et actuel du Léopard ocellé (*Lacerta lepida*, Sauriens, Lacertidae) en France. Implication en termes de conservation. *Vie et Milieu* 55(1): 15–30.
- Congedo L, Sallustio L, Munafo M, Ottaviano M, Tonti D, Marchetti M. 2016. Copernicus high-resolution layers for land cover classification in Italy. *Journal of Maps* 12(5): 1,195–1,205.
- Cutler DR, Edwards TC Jr, Beard KH, Cutler A, Hess KT, Gibson J, Lawler JJ. 2007. Random forests for classification in ecology. *Ecology* 88(11): 2,783–2,792.
- Delibes-Mateos M, Delibes M, Ferreras P, Villafuerte R. 2008. Key role of European rabbits in the conservation of the western Mediterranean basin hotspot. *Conservation Biology* 22: 1,106–1,117.
- Deso G, Dusoulier F, Bence S, Cheylan M. 2011. Distribution du Léopard ocellé *Timon lepidus lepidus* (Daudin, 1801) dans la Haute vallée de la Durance (Région Provence-Alpes-Côte-d’Azur). *Bulletin de la Société Herpétologique de France* 137: 43–50.
- Deso G, Cevasco J-M, Salvidio S, Ottonella D, Oneto F, Cheylan M. 2015. Statut des populations franco-italiennes de Léopard ocellé *Timon lepidus lepidus* (Daudin, 1801). *Bulletin de la Société Herpétologique de France* 156: 45–53.
- Doorman CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, Garcia-Marquez JR, Gruber B, Lafourcade B, Leitao PJ, et al. 2012. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography* 36: 27–46.
- Doré F, Cheylan M, Grillet P. 2015. *Le Léopard Ocellé. Un Géant sur le Continent Européen*. Biotope, Mèze, France. 192 p.
- Drake JM, Randin C, Guisan A. 2006. Modelling ecological niches with support vector machines. *Journal of Applied Ecology* 43: 424–432.
- Elith J, Graham CH, Anderson RP, Dudik M, Ferrier S, Guisan A, Hijmans RJ, Huettmann F, Leathwick JR, Lehmann A, et al. 2006. Novel methods improve prediction of species’ distributions from occurrence data. *Ecography* 29(2): 129–151.
- Elith J, Leathwick J. 2007. Predicting species distributions from museum and herbarium records using multiresponse models fitted with multivariate adaptive regression splines. *Diversity and Distribution* 13: 265–275.
- Elith J, Leathwick JR, Hastie T. 2008. A working guide to boosted regression trees. *Journal of Animal Ecology* 77: 802–813.
- Elith J, Leathwick JR. 2009. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics* 40: 677–697.
- Engler R, Guisan A, Rechsteiner L. 2004. An improved approach for predicting the distribution of rare and endangered species from occurrence and pseudo-absence data. *Journal of Applied Ecology* 41(2): 263–274.
- Ferreira AF, Quintella BR, Maia C, Mateus CS, Alexandre CM, Capinha C, Almeida PR. 2013. Influence of macrohabitat preferences on the distribution of European brook and river lampreys: Implications for conservation and management. *Biological Conservation* 159: 175–186.
- Ferrier S. 2002. Mapping spatial pattern in biodiversity for regional conservation planning: where to from here? *Systematic Biology* 51: 331–363.
- Ferrier S, Watson G, Pearce J, Drielsma M. 2002. Extended statistical approaches to modelling spatial pattern in biodiversity in northeast New South Wales. I. Species-level modelling. *Biodiversity and Conservation* 11: 2,275–2,307.
- Fick SE, Hijmans RJ. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 37(12): 4,302–4,315.
- Fielding AH, Bell JF. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation* 24(1): 38–49.
- Fourcade Y, Besnard A, Secondi J. 2018. Paintings predict the distribution of species, or the challenge of selecting environmental predictors and evaluation

- statistics. *Global Ecology and Biogeography* 27(2): 245–256.
- Franklin J. 1998. Predicting the distribution of shrub species in southern California from climate and terrain-derived variables. *Journal of Vegetation Science* 9: 733–748.
- Franklin J. 2009. *Mapping Species Distributions, Spatial Inference, and Prediction*. Cambridge University Press, Cambridge, United Kingdom. 320 p.
- Geniez P, Cheylan M. 2012. *Les Amphibiens et les Reptiles du Languedoc-Roussillon et Régions Limitrophes. Atlas Biogéographique*. Biotope, Mèze, France, and Muséum National d'Histoire Naturelle, Paris, France. 448 p.
- GHRA-LPO Rhône-Alpes (2015). *Les Amphibiens et Reptiles de Rhône-Alpes*. LPO coordination Rhône-Alpes, Lyon, France. 448 p.
- Graham CH, Ferrier S, Huetteman F, Moritz C, Peterson AT. 2004. New developments in museum-based informatics and applications in biodiversity analysis. *Trends in Ecology and Evolution* 19(9): 497–503.
- Griffith B, Scott JM, Carpenter JW, Reed C. 1989. Translocation as a species conservation tool: status and strategy. *Science* 245: 477–480.
- Grillet P, Cheylan M, Dusoulrier F. 2006. Evolution des habitats et changements climatique: quelles conséquences pour les populations de lézard ocellé, *Lacerta lepida* (Saurien, Lacertidés) en limite nord de distribution? *Ecologia Mediterranea* 32: 63–72.
- Grillet P, Cheylan M, Thirion JM, Doré F, Bonnet X, Dauge C, Chollet S, Marchand MA. 2010. Rabbit burrows or artificial refuges are a critical habitat component for the threatened lizard *Timon lepidus* (Sauria, Lacertidae). *Biodiversity and Conservation* 19(7): 2,039–2,051.
- Guisan A, Zimmermann NE. 2000. Predictive habitat distribution models in ecology. *Ecological Modelling* 135(2–3): 147–186.
- Guisan A, Hofer U. 2003. Predicting reptile distributions at the mesoscale: relation to climate and topography. *Journal of Biogeography* 30(8): 1,233–1,243.
- Guisan A, Thuiller W. 2005. Predicting species distribution: offering more than simple habitat models. *Ecology Letters* 8(9): 993–1,009.
- Guisan A, Tingley R, Baumgartner JB, Naujokaitis-Lewis I, Sutcliffe PR, Tulloch AIT, Regan TJ, Brotons L, McDonald-Madden E, Mantyka-Pringle C, et al. 2013. Predicting species distributions for conservation decisions. *Ecology Letters* 16(12): 1,424–1,435.
- Harvey PH, Colwell R, Silvertown J, May RM. 2003. Null models in ecology. *Annual Review of Ecology and Systematics* 14(1): 189–211.
- Heikkinen RK, Luoto M, Araújo MB. 2006. Methods and uncertainties in bioclimatic envelope modelling under climate change. *Progress in Physical Geography* 30(6): 751–777.
- Hirzel A, Guisan A. 2002. Which is the optimal sampling strategy for habitat suitability modelling? *Ecological Modelling* 157: 331–341.
- Huete A, Didan K, van Leeuwen W, Miura T, Glenn E. 2010. MODIS vegetation indices. Pp. 579–602 In: *Land Remote Sensing and Global Environmental Change. Remote Sensing and Digital Image Processing, Volume II*. Editors, Ramachandran B, Justice C, Abrams M. Springer, New York, New York, USA. 873 p.
- Humboldt A, Bonpland A. 1805. *Essai sur la Géographie des Plantes accompagné d'un Tableau Physique des Régions Équinoxiales*. [Volume] XIII. Levrault, Schoell et Compagnie, Paris, France. 155 p.
- Iannella M, Cerasoli F, D'Alessandro P, Console G, Biondi M. 2018. Coupling GIS spatial analysis and ensemble niche modelling to investigate climate change-related threats to the Sicilian pond turtle *Emys trinacris*, an endangered species from the Mediterranean. *PeerJ* 6: e4969.
- Jiang G, Sun H, Lang J, Yang L, Li C, Lyet A, Long B, Miquelle DG, Zhang C, Aramilev S, et al. 2014. Effects of environmental and anthropogenic drivers on Amur tiger distribution in northeastern China. *Ecological Research* 29(5): 801–813.
- Joly D, Brossard T, Cardot H, Cavailles J, Hilal M, Wavresky P. 2010. Les types de climats en France, une construction spatiale. *Cybergeo* 501: 1–23.
- Karger DN, Conrad O, Böhner J, Kawohl T, Kreft H, Soria-Auza RW, Zimmermann NE, Linder HP, Kessler M. 2017. Climatologies at high resolution for the Earth land surface areas. *Scientific Data* 4: 170122.
- Leguëdois S, Party J-P, Dupouey J-L, Gauquelin T, Gégout J-C, Lecareux C, Badeau V, Probst A. 2011. La carte de végétation du CNRS à l'ère du numérique. *European Journal of Geography* 559: 1–36.
- Li X, Wang Y. 2012. Applying various algorithms for species distribution modelling. *Integrative Zoology* 8(2): 124–135.
- Liu C, White M, Newell G. 2009. Measuring the accuracy of species distribution models: a review. Pp. 4,241–4,247 In: *18th World IMACS/ MODSIM Congress, Cairns, Australia 13-17 July 2009*. Editors, Anderssen RS, Braddock RD, Newham LTH. Modelling and Simulation Society of Australia and New Zealand, Canberra, Australia.
- Lyet A, Thuiller W, Cheylan M, Besnard A. 2013. Fine-scale regional distribution modelling of rare and threatened species: bridging GIS tools and conservation in practice. *Diversity and Distributions* 19(7): 651–663.
- Manel S, Dias J-M, Ormerdo S. 1999. Comparing discriminant analysis, neural networks, and logistic regression for predicting species distributions: a case study with a Himalayan river bird. *Ecological Modelling* 120: 337–347.
- Mateo JA. 2011. Lagarto ocelado - *Timon lepidus*. In: *Enciclopedia Virtual de los Vertebrados Españoles*.

- Editors, Salvador A, Marco A. Museo Nacional de Ciencias Naturales, Madrid, Spain. Available: <http://www.vertebradosibericos.org/reptiles/timlep.html> [Accessed: 14 October 2018].
- Miraldo A, Faria C, Hewitt GM, Paulo OS, Emerson BC. 2013. Genetic analysis of a contact zone between two lineages of the Ocellated Lizard (*Lacerta lepida* Daudin 1802) in south-eastern Iberia reveal a steep and narrow hybrid zone. *Journal of Zoological Systematics and Evolutionary Research* 51(1): 45–54.
- Moradi S, Ilanloo SS, Kafash A, Yousefi M. 2019. Identifying high-priority conservation areas for avian biodiversity using species distribution modelling. *Ecological Indicators* 97: 159–164.
- Muñoz AR, Real R, Barbosa AM, Vargas JM. 2005. Modelling the distribution of Bonelli's eagle in Spain: implication for conservation planning. *Diversity and Distributions* 11: 477–486.
- Naimi B, Araújo MB. 2016. Sdm: a reproducible and extensible R platform for species distribution modelling. *Ecography* 39: 368–375.
- Naimi B, Hamm NA, Groen TA, Skidmore AK, Toxopeus AG. 2014. Where is positional uncertainty a problem for species distribution modelling. *Ecography* 37: 191–203.
- Nenzen HK, Araújo MB. 2011. Choice of threshold alters projections of species range shifts under climate change. *Ecological Modelling* 222: 3,346–3,354.
- Poitevin F, Olivier A, Bayle P, Scher O. 2010. *Mammifères de Camargue*. Regard du Vivant et Parc Naturel Régional de Camargue, Castelnau-le-Lez, France. 232 p.
- Pottier G, Cochard P-O, Barthe L, Cheylan M, Geniez P, Defos du Rau P. 2017. État des connaissances sur la répartition du Léopard ocellé *Timon lepidus* (Daudin, 1802) dans l'ouest de la région Occitanie (ancienne région Midi-Pyrénées). *Bulletin de la Société Herpétologique de France* 161: 23–56.
- Prodon R, Geniez P, Cheylan M, Devers F, Chuine I, Besnard A. 2017. A reversal of the shift towards earlier spring phenology in several Mediterranean reptiles and amphibians during the 1998–2013 warming slowdown. *Global Change Biology* 12: 5,481–5,491.
- Pulliam HR. 2000. On the relationship between niche and distribution. *Ecology Letters* 3: 349–361.
- Raes N, ter Steege H. 2007. A null-model for significance testing of presence-only species distribution models. *Ecography* 30(5): 727–736.
- Rangel TF, Loyola RD. 2012. Labeling ecological niche models. *Natureza & Conservação* 10(2): 119–126.
- Raxworthy CJ, Martinez-Meyer E, Horning N, Nussbaum RA, Schneider GE, Ortega-Huerta MA, Peterson AT. 2003. Predicting distributions of known and unknown reptile species in Madagascar. *Nature* 426: 837–841.
- Renwick KM, Curtis C, Kleinhesselink AR, Schlaepfer D, Bradley BA, Aldridge CL, Poulter B, Adler PB. 2018. Multi-model comparison highlights consistency in predicted effect of warming on a semi-arid shrub. *Global Change Biology* 24: 424–438.
- Ryberg WA, Wolaver BD, Prestridge HL, Labay BJ, Pierre JP, Costley RA, Adams S, Bowers BC, Hibbitts TJ. 2017. Habitat modelling and conservation of the Western Chicken Turtle (*Dierochelys reticularia miara*). *Herpetological Conservation and Biology* 12(2): 307–320.
- Salas EAL, Seamster VA, Harings NM, Boykin KG, Alvarez G, Dixon KW. 2017. Projected future bioclimate-envelope suitability for reptile and amphibian species of concern in South Central USA. *Herpetological Conservation and Biology* 12(2): 522–547.
- Salvidio S, Lamagni L, Bombi P, Bologna MA. 2004. Distribution, ecology, and conservation status of the Ocellated Lizard (*Timon lepidus*) in Italy (Reptilia, Lacertidae). *Bollettino di Zoologia* 71: 125–134.
- Santos X, Brito JC, Caro J, Abril AJ, Lorenzo M, Sillero N, Pleguezuelos JM. 2009. Habitat suitability, threats, and conservation of isolated populations of the smooth snake (*Coronella austriaca*) in the southern Iberian Peninsula. *Biological Conservation* 142(2): 344–352.
- Santos X, Cheylan M. 2013. Taxonomic and functional response of a Mediterranean reptile community to a repeated fire regime. *Biological Conservation* 168: 90–98.
- Sillero N. 2011. What does ecological modelling model? A proposed classification of ecological niche models based on their underlying methods. *Ecological Modelling* 222: 1,343–1,346.
- Sillero N, Carretero MA. 2013. Modelling the past and future distribution of contracting species. The Iberian lizard *Podarcis carbonelli* (Squamata: Lacertidae) as a case study. *Zoologischer Anzeiger* 252: 289–298.
- Sillero N, Campos J, Bonardi A, Corti C, Creemers R, Crochet PA, Crnobrnja Isailović J, Denoël M, Ficetola GF, Gonçalves J, et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe. *Amphibia-Reptilia* 35(1): 1–21.
- Sohrab M, Sayyad SI, Anooshe K, Masoud Y. 2019. Identifying high-priority conservation areas for avian biodiversity using species distribution modeling. *Ecological Indicators* 97: 159–164.
- Souter NJ, Bull CM, Lethbridge MR, Hutchinson MN. 2007. Habitat requirements of the endangered Pygmy Bluetongue Lizard, *Tiliqua adelaidensis*. *Biological Conservation* 135(1): 33–45.
- Thuiller W, Araújo MB, Lavorel S. 2004. Do we need land-cover data to model species distributions in Europe? *Journal of Biogeography* 31: 353–361.
- Vetaas OR. 2002. Realized and potential climate niches: a comparison of four *Rhododendron* tree species. *Journal of Biogeography* 29: 545–554.
- Wan J, Wang C, Yu J, Nie S, Han S, Liu J, Zu Y, Wang Q. 2016. Developing conservation strategies for *Pinus koraiensis* and *Eleutherococcus senticosus* by using

model-based geographic distributions. *Journal of Forestry Research* 27(2): 389–400.

Wang CJ, Wan JZ, Zhang ZX, Zhang GM. 2016. Identifying appropriate protected areas for endangered fern species under climate change. *SpringerPlus* 5(1): 904.

Ward D. 2005. *Reversing Rabbit Decline: One of the Biggest Challenges for Nature Conservation in Spain and Portugal*. Field Reports. IUCN/SSC, Lagomorph Specialist Group, Gland, Switzerland. 54 p.

Wilson JW, Sexton JO, Jobe RT, Haddad NM. 2013. The relative contribution of terrain, land cover, and vege-

tation structure indices to species distribution models. *Biological Conservation* 164: 170–176.

Wood SN, Pya N, Säfken B. 2016. Smoothing parameter and model selection for general smooth models. *Journal of the American Statistical Association* 111: 1,548–1,563.

Zomer RJ, Trabucco A, Bossio DA, van Straaten O, Verchot LV. 2008. Climate change mitigation: A spatial analysis of global land suitability for clean development mechanism afforestation and reforestation. *Agriculture Ecosystems and Environment* 126: 67–80.



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Distribution of *Timon lepidus* in France



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First field report of *Trimerodytes percarinatus* (Boulenger, 1899) (Reptilia: Squamata: Natricidae) from India with notes on its natural history

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The Asian snake genus *Trimerodytes* Cope, 1895 belongs to the family Natricidae and consists of four species, namely *Trimerodytes annularis* (Hallowell, 1856), *Trimerodytes percarinatus* (Boulenger, 1899), *Trimerodytes aequifasciatus* (Barbour, 1908), and *Trimerodytes yunnanensis* (Rao and Yang, 1998). *Trimerodytes percarinatus* (Boulenger, 1899) is a nocturnal snake commonly known as the Eastern Water Snake or Chinese Keelback Water Snake. This species inhabits water passages in forested hilly country (100–2,000 m asl) and generally feeds on fish, crayfish, crustaceans, frogs, and their larvae (Pope 1935; Smith 1943). Pope (1935) also recorded its presence in irrigated fields near a forest in Kuatun, China. Currently, *Trimerodytes percarinatus* has two recognized subspecies, *Trimerodytes percarinatus percarinatus* (Boulenger, 1899) and *Trimerodytes percarinatus suriki* (Maki, 1931).

Trimerodytes percarinatus was originally described as *Tropidonotus percarinatus* by Boulenger in 1899, from Kuatun, Foochow, in the north-west of the Province of Fokien (=Fujian), China at an altitude of 3,000–4,000 ft or more (Zhao and Adler 1993). Subsequently, it was placed within the genus *Natrix* by Mell (1931) and later assigned to the genus *Sinonatrix* by Rossman and Eberle (1977). Based on a recent phylogenetic study, it is placed within the genus *Trimerodytes* (Ren et al. 2019).

This species is distributed in north-eastern India (Arunachal Pradesh), Myanmar, Thailand, Laos, Vietnam, south-eastern China, and Taiwan at elevations ranging from 90–2,000 m (Pope 1935; Smith 1943; Taylor 1965; Zhao and Adler 1993; Stuart and Heatwole 2008; Nguyen et al. 2009; Boundy et al. 2014). Captain

and Patel (1998) first reported the existence of the genus *Trimerodytes* in India, represented by this species, *T. percarinatus*, based on an uncatalogued museum specimen housed previously at Deban Forest Camp and now at the Namdapha Tiger Reserve Field Museum in Miao, India. For the next two decades since then, though studies documenting the herpetofauna of Arunachal Pradesh have been conducted, this species has not been recorded (Athreya et al. 1998; Borang et al. 2005; David and Mathew 2005; Agarwal et al. 2010). Here, we report the first field record of *Trimerodytes percarinatus* (Boulenger, 1899) from India with notes on its natural history.

This species was recorded on two occasions in Namdapha Tiger Reserve, Arunachal Pradesh, India. This national park harbors a rich biodiversity and is part of one of the world's biodiversity hotspots (Indo-Myanmar). The first individual (Fig. 1) was encountered at 27°28'58"N, 96°24'14"E and an elevation of 515 m asl, at approximately 2200 h on 17 June 2011. It was found inside a small ditch filled with water (~1 ft deep) near the road edge, with its head out of the water and it was foraging actively. The second individual was recorded at approximately 1730 h on 18 June 2011. It was foraging in a water passage near the edge of a road. A *Fowlea* cf. *piscator* was also seen foraging in the same water body. The first individual was captured for morphological measurements and photographs, and was later released into the wild. A detailed description of the specimen is given in Table 1, and follows the methods from Vogel et al. (2004). Comparisons between the left and right sides of the head, and associated habitat where the snake was encountered, are also presented (Figs. 2–4).

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Fig. 1 Full body picture of *Trimerodytes percarinatus* from Namdapha, India.



Fig. 3 Habitat of *Trimerodytes percarinatus* at Namdapha, India.

Morphometric data for this specimen fall within the range of *Trimerodytes percarinatus* as defined in the available literature (Pope 1935; Smith 1943). These observations provide two additional records of *Trimerodytes percarinatus* from India and the first field report from the country, though the sightings were in more or less the same place (“Deban”) associated with the earlier specimen (see Captain and Patel 1998). This work, as well as other literature on this genus including new range records of other congeners from Indochina (Vogel et al. 2004; Pauwels et al. 2009; Le et al. 2015), points out our incomplete understanding of the distribution of this genus as a whole. Further work is required to determine the actual distribution range of this species in India, to understand its morphological and genetic variation across populations, and to add to our knowledge of its natural history.

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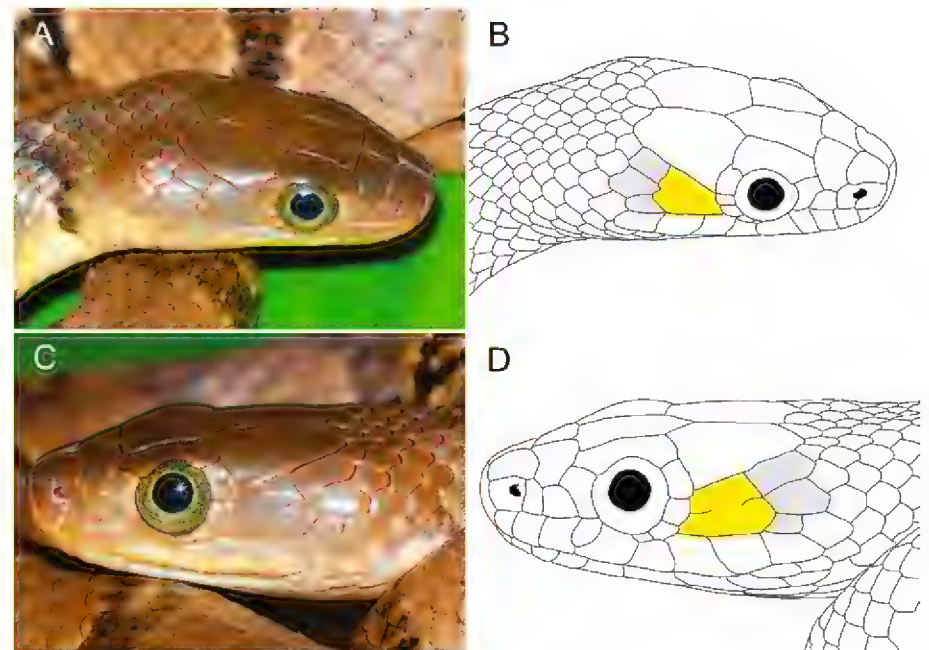


Fig. 2 Comparison between right and left sides of the head of *Trimerodytes percarinatus* from Namdapha, India.

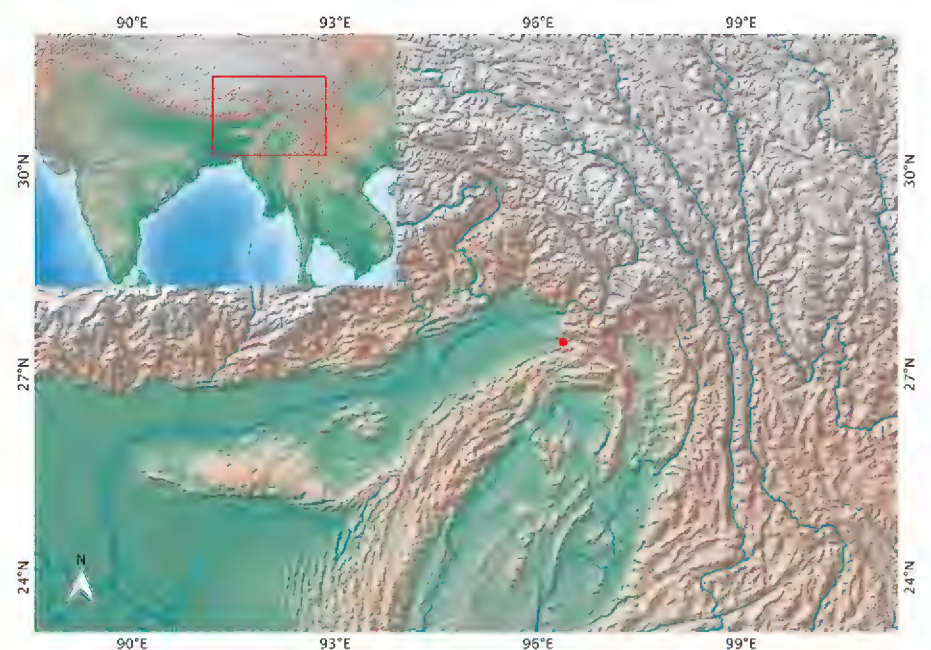


Fig. 4 Location of first field sighting of *Trimerodytes percarinatus* in Namdapha Tiger Reserve, Arunachal Pradesh, India.

in protected areas of Arunachal Pradesh. We thank Kartik Shanker for his valuable input to our manuscript, and for providing financial and logistic support during our fieldwork. Also, many thanks to the Field Director, Assistant Field Director Dr. Aporesh Gupta-Choudhury, and other forest staff from Deban guest house, Namdapha Tiger Reserve, for their hospitality during the fieldwork.

Literature Cited

- Agarwal I, Mistry VK, Athreya RM. 2010. A preliminary checklist of the reptiles of Eaglenest Wildlife Sanctuary, West Kameng District, Arunachal Pradesh, India. *Russian Journal of Herpetology* 17: 81–93.
- Athreya RM, Captain AS, Athreya VR. 1997. *A Faunal Survey of Namdapha Tiger Reserve Arunachal Pradesh, India: Notes on Some of the More Interesting Species*. Unpublished report. Arunachal Pradesh Forest Department. Itanagar, Arunachal Pradesh, India.
- Borang A, Bhatt BB, Chaudhury SB, Borkotoki A, Bhutia PT. 2005. Checklist of the snakes of Arunachal Pradesh, northeast India. *Journal of Bombay Natural History Society* 102(1): 19–26.

- Captain A, Patel A. 1998. *Sinonatrix*, a new genus for India. *Hamadryad* 22(2): 114–115.
- Cope ED. 1895. On a collection of Batrachia and Reptilia from the Island of Hainan. *Proceedings of the Academy of Natural Science of Philadelphia* XLVI: 423–428.
- David P, Mathew R. 2004. Notes on some noteworthy snake specimens deposited in the collections of Eastern Regional Station of the Zoological Survey of India. *Records of the Zoological Survey of India* 104(3-4): 83–90.
- Le DT, Pham AV, Pham CT, Nguyen SHL, Ziegler T, Nguyen TO. 2015. Review of the genus *Sinonatrix* in Vietnam with a new country record of *Sinonatrix yunnanensis* Rao et Yang, 1998. *Russian Journal of Herpetology* 22(2): 84–88.
- Mell R. 1931. List of Chinese snakes. *Lingnan Science Journal* 8: 199–219.
- Nguyen VS, Ho TC, Nguyen QT. 2009. *Herpetofauna of Vietnam*. Edition Chimaira, Frankfurt, Germany. 768 p.
- Pauwels OSG, Kunya K, David P, Sumontha M. 2009. First record of the Yunnan Keelback *Sinonatrix yunnanensis* Rao and Yang, 1998 (Serpentes: Natricidae) from Thailand. *Salamandra* 45(3): 165–169.
- Pope CH. 1935. *The Reptiles of China*. Pp. 116–120 In: *Natural History of Central Asia*. Volume X. American Museum of Natural History, New York, New York, USA. 604 p.
- Ren J, Wang K, Guo P, Wang Y, Nguyen TT, Li J. 2019. On the generic taxonomy of *Opisthotropis balteata* (Cope, 1895) (Squamata: Colubridae: Natricinae): taxonomic revision of two natricine genera. *Asian Herpetological Research* 10(2): 105–128.
- Rossman DA, Eberle WG. 1977. Partition of the genus *Natrix*, with preliminary observations on evolutionary trends in natricine snakes. *Herpetologica* 1: 34–43.
- Smith MA. 1943. *The Fauna of British India, Ceylon, and Burma, including the Whole of the Indo-Chinese Sub Region*. Reptilia and Amphibia, Volume 3 (Serpentes). Taylor and Francis, London, United Kingdom. 583 p.
- Stuart BL, Heatwole H. 2008. Country records of snakes from Laos. *Hamadryad* 33: 97–106.
- Taylor EH. 1965. The serpents of Thailand and adjacent waters. *The University of Kansas Science Bulletin* 45(9): 609–1,096.
- Vogel G, David P, Pauwels OSG, Brachtel N. 2004. On the occurrence of the watersnake *Sinonatrix aequifasciata* (Barbour, 1908) (Serpentes, Colubridae, Natricinae) in Vietnam. *Hamadryad* 29(1): 110–114.
- Wallach V, Williams KL, Boundy J. 2014. *Snakes of the World: a Catalogue of Living and Extinct Species*. Taylor and Francis/CRC Press, Boca Raton, Florida, USA. 677 p.
- Zhao E, Adler K. 1993. *Herpetology of China*. Contributions to Herpetology, Volume 10. Society for the Study of Amphibians and Reptiles in cooperation with Chinese Society for the Study of Amphibians and Reptiles, Oxford, Ohio, USA. 522 p.
- Zhao E. 1986. Partition of Chinese *Natrix* species and a suggestion of their Chinese names. *Acta Herpetologica Sinica* 5(3): 239–240. [in Chinese, English summary]

Table 1. Characteristics of one specimen of *Trimerodytes percarinatus* from Namdapha, Arunachal Pradesh, India, compared with descriptions in Pope (1935) and Smith (1943).

Characters	This study	Pope (1935)	Smith (1943)
Dorsal scale rows	19:19:17, keeled	19	19
Ventrals	153	138–143	133–157
Subcaudals	69/70	70–79 (males), 67–73 (females)	68–85
Anal	Divided	Divided	–
Snout-vent length (mm)	433 (male)	515–567 (males); 620–730 (females)	720 (male); 940 (female)
Tail length (mm)	131	130	190 (male); 270 (female)
Head length (mm)	22.1	–	–
Horizontal eye diameter (mm)	3.9	–	–
Vertical eye diameter (mm)	3.9	–	–
Eye to nasal distance (mm)	4.0	–	–
Eye-snout distance (mm)	6.8	–	–
Inter-nasal distance (mm)	4.8	–	–
Prefrontal (mm)	2.6	–	–
Parietal (mm)	7.3	–	–
Length of supraocular (mm)	5.3	–	–
Width of supraocular (mm)	2.7	–	–

Table 1 (continued). Characteristics of one specimen of *Trimerodytes percarinatus* from Namdapha, Arunachal Pradesh, India, compared with descriptions in Pope (1935) and Smith (1943).

Characters	This study	Pope (1935)	Smith (1943)
Supralabials	8 (left), 4 th touching eye / 9 (right), 4 th and 5 th touching eye	9 (rarely 8 or 10), 4 th and 5 th entering the eye	9, 4 th and 5 th touching eye
Infralabials	10 (on left and right); 1 st –5 th touch anterior genials (chin shields); 5 th and 6 th touch posterior genials – which are longer than anterior genials	5 lower labials in contact with anterior chin shields that are shorter than posterior	–
Preoculars	1 (+1 presubocular)/ 1	single	1
Postoculars	3 (+1 postsubocular/ 3 (+2 postsuboculars)	4 (occasionally 3 or 5)	–
Supraocular	1/ 1	–	–
Temporals	1 (2 fused scales) + 3/ 1 (2 fused scales) + 3	3 (occasionally 2 or 4)/ 3 (occasionally 2, rarely 4)	2+3 (rarely 3+3)
Cross bands (on body)	30	–	–
Nostril	Directed upward	–	Directed slightly upward
Nasal	Partially divided	Completely divided	–
Internasal	Narrowed anteriorly, longer than the prefrontal	Narrowed anteriorly, longer than broad, and longer than prefrontal	Distinctly narrowed anteriorly, usually longer than the prefrontal
Prefrontal	Broader than long	Shorter than internasal	–
Frontal	Long and pointed posteriorly	As long as broad, as long as its distance from the end of snout, shorter than parietals	–
Coloration	Dorsum greyish brown with uniform cross-bars, dorsolateral portion light yellow in color, venter whitish with uniform paired and unpaired bars along with light yellowish ventrolateral	Dorsum greyish olive, sides with light-edged black vertical bars; venter uniform yellowish white anteriorly, spotted and speckled with blackish posteriorly; lower surface of tails dark grey in color along with black spots	Young: dorsum grey or dark olive green, color descending on the dorsolateral side as V shaped bars, the interval between bars and lower portion yellowish; Adults: dorsum greyish or olivaceous with uniform or with dark reticulation or dark cross-bars, venter whitish, with or without dark cross-bars



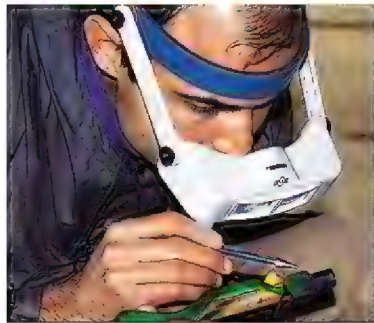
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Ashok Captain is a renowned Indian herpetologist who is interested in and dabbles with the traditional taxonomy of snake species that occur in India.



The most frog-diverse place in Middle America, with notes on the conservation status of eight threatened species of amphibians

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Abstract.—Regarding amphibians, Costa Rica exhibits the greatest species richness per unit area in Middle America, with a total of 215 species reported to date. However, this number is likely an underestimate due to the presence of many unexplored areas that are difficult to access. Between 2012 and 2017, a monitoring survey of amphibians was conducted in the Central Caribbean of Costa Rica, on the northern edge of the Matama mountains in the Talamanca mountain range, to study the distribution patterns and natural history of species across this region, particularly those considered as endangered by the International Union for Conservation of Nature. The results show the highest amphibian species richness among Middle America lowland evergreen forests, with a notable anuran representation of 64 species. The greatest diversity in the study area occurred in the mature forest on the basal belt. Of the 68 amphibian species found, seven (10%) are endemic to the Atlantic versant and eight (11.6%) are threatened. This survey includes the first record of *Gastrotheca cornuta* in Costa Rica since it was last reported 21 years ago. New populations of *Agalychnis lemur* (Critically Endangered) and *Duellmanohyla uranochroa* (Endangered) are reported, and *Ecnomiohyla veraguensis* (Endangered) is reported for the first time in Costa Rica. These findings show that this locality is a high priority conservation area for a large number of amphibian species, which are often threatened by habitat loss and fragmentation.

Keywords. Biodiversity, Costa Rica, Endangered, Limón province, patterns of distribution, Tropical Wet Forest

Resumen.—En anfibios, Costa Rica exhibe la mayor riqueza de especies por unidad de área en América Meridional con un total 215 especies documentadas a la fecha. Sin embargo, es probable que este número esté subestimado debido a la presencia de áreas inexploradas con difícil acceso. Entre 2012 y 2017, realizamos un monitoreo de anfibios en el Caribe Central de Costa Rica, en el borde norte de la Fila Matama en la Cordillera de Talamanca, para estudiar los patrones de distribución y la historia natural de las especies en esta región, particularmente aquellas consideradas en peligro por la Unión Internacional para la Conservación de la Naturaleza (UICN). Nuestros resultados muestran la mayor riqueza de especies de anfibios en los bosques perennes de tierras bajas de América Meridional, con una notable representación de anuros de 64 especies. La mayor diversidad en el área de estudio se encontró en el bosque maduro en el piso basal. Del total de especies, siete (10%) son endémicas de la vertiente Atlántico y ocho (11,6%) están amenazadas. Este es el primer registro de *Gastrotheca cornuta* en Costa Rica después de 21 años desde que se registró por última vez. Descubrimos nuevas poblaciones de *Agalychnis lemur* (en Peligro Crítico), *Duellmanohyla uranochroa* (en Peligro), y reportamos por primera vez *Ecnomiohyla veraguensis* (en Peligro) en Costa Rica. Nuestros resultados muestran que esta localidad es un área de alta prioridad para la conservación de una gran cantidad de especies de anfibios, a menudo amenazadas por la fragmentación y la pérdida de hábitat.

Palabras clave. Biodiversidad, Costa Rica, amenazado, provincia de Limón, patrones de distribución, Bosque Tropical Húmedo

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Introduction

Currently, more than 8,000 species of amphibians have been described worldwide, with the greatest diversity occurring in the Neotropics (Duellman 1999a; Frost 2019), where lower Central America stands out as a region with a substantial number of species (Campbell 1999; Duellman 2001; Savage 2002; Kubicki 2007). However, there has been an increase in the numbers of species designated as endangered throughout this region since the late 1980s due to habitat deforestation (Young et al. 2001; Stuart et al. 2004; Becker et al. 2007), climate change (Pounds et al. 1999; Hof et al. 2011), and infectious diseases (Lips et al. 2003; Pounds et al. 2006; Wake and Vredenburg 2008).

Tropical forests harbor a considerable number of amphibian species across distinct microhabitats that are often related to water-dependent sites such as ponds, temporary swamps, streams, tree holes, and bromeliad axils (Duellman 1970; Savage 2002; Lehtinen et al. 2004; Haddad and Prado 2005). However, many other amphibians exhibit reproductive modes that are totally independent from water bodies (Savage 2002). For instance, frogs of the genera *Gastrotheca* (Hemiphractidae), *Eleutherodactylus* (Eleutherodactylidae), *Incilius* (formerly *Crepidophryne*: Bufonidae), *Craugastor*, *Pristimantis*, and *Strabomantis* (Craugastoridae) lay encapsulated eggs out of water, in which embryos undergo direct development and hatch out as small adults (Savage 2002; Gray and Bland 2016).

Costa Rica is known to possess considerable amphibian species richness per unit area (Savage 2002; Sasa et al. 2010; Bolaños et al. 2011). However, this great richness is likely to be underestimated due to the presence of undiscovered and undescribed species in areas that tend to be not easily accessible. To date, several previous works have reported countrywide amphibian checklists (Savage 2002; Bolaños et al. 2011; Leenders 2016). In particular, an increasing interest has focused on documenting species occurrence and the population status of threatened species in Costa Rica's montane ecosystems (Hayes et al. 1989; Abarca 2012; Acosta-Chaves et al. 2015; Rovito et al. 2015) and tropical forests of varying altitudinal gradients in the Pacific Slope throughout its South (McDiarmid and Savage 2005), Central (Laurencio and Malone 2009), and Northern regions (Sasa and Solorzano 1995). In contrast, the amphibian diversity of the Costa Rican Atlantic has been broadly documented almost exclusively in its Northern region (Donnelly and Guyer 1994; Guyer and Donnelly 2005; Whitfield et al. 2007). Only recently, Kubicki (2008) compiled the first list of species in premontane moist forests of the Costa Rican central-Caribbean. Although that inventory showed the relevant diversity of amphibians, little is known about the current population status and distribution of amphibian species across other areas of the mid-Caribbean.

Along this region, the Talamanca mountain range stands out as a predicted high priority conservation area for amphibians (García-Rodríguez et al. 2011). Considering that long-term monitoring can accurately assess population conditions (La Marca et al. 2005), species inventories are key to covering gaps in the distribution and natural history of endangered species in order to determine appropriate conservation strategies (Peloso 2010; Verdade et al. 2012). This study assessed the local richness and species distribution of amphibians in Veragua Rainforest Eco Research and Adventure Park and its surroundings on the northern edge of the Matama mountains in the Talamanca mountain range. Diversity analysis was conducted for different types of altitudinal belts, forests, and microhabitats found across the sampling area, and the population status of the threatened species in the study area are discussed.

Materials and Methods

Study area. The study was conducted in the Central Caribbean of Costa Rica between Las Brisas de Veragua town (9°57'07"N, 83°12'11"E; 233 m asl) and Plátano peak (9°51'50"N, 83°14'10"E; 1,000 m asl) on the northern edge of the Matama mountains in the Talamanca mountain range, including Chimú peak (9°52'48"N, 83°14'13"E; 741 m asl) and Veragua Rainforest Park (VRP; 9°55'30"N, 83°11'28"E; 420 m asl; Fig. 1). This private reserve covers 3,200 ha of protected land ranging from 200–420 m asl, and it comprises mature forest, secondary vegetation at different stages of regeneration, open areas, and dirt roads. The study site lies adjacent to Victoria (9°55'21.73"N, 83°10'2.43"E; 410 m asl) on the Victoria river basin and the Matama mountains. This area is the closest point of the Talamanca mountain range to the Caribbean Sea and it forms part of the buffer zones of La Amistad International Park (an UNESCO World Heritage Site), the Banano river basin protected area, and the influence zones of the Zent, Peje, and Chirripó rivers, as well as the Bajo Chirripó indigenous reserve (SINAC 2018). Sampling was carried out along the elevation range 200–1,000 m asl, where two types of forest are located according to Holdridge (1967): Basal Tropical Wet Forest (200–600 m asl) and Premontane Tropical Wet Forest (601–1,000 m asl). Only these altitudinal belts are recorded and reported here, because they both represent the Tropical Wet Forest.

Data collection. Data were collected between January 2012 and December 2017. To record the species richness, samplings were standardized through diurnal and nocturnal visual and acoustic recognition searches (Crump and Scott 1994) into three transects: two in VRP, covering approximately 4 km each (Transects A and B), and one carried out along an 11 km trail between the reserve and Plátano peak (Transect C), including Chimú peak halfway along the route. On each of these

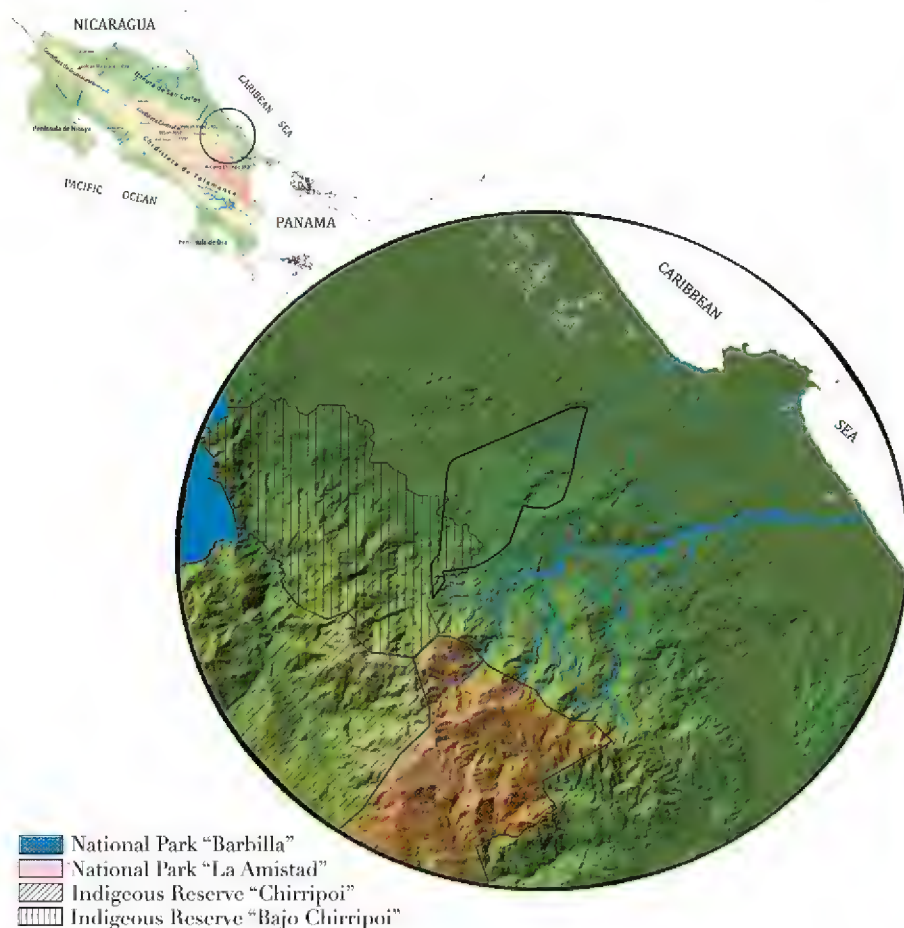


Fig. 1. Location of the study area (51 km²) in the Limón Province in the Central Caribbean area of Costa Rica. The colored points represent the main localities of the study area: yellow (Chimú Peak), red (Plátano Peak), blue (Veragua Rainforest Research and Adventure Park), green (Las Brisas), and purple (Victoria).

transects, surveyors walked side-by-side at a constant speed to record amphibian diversity on both sides of the trail (Seber 1986), covering up to 10 m from each side towards the forest. Transect A (300–420 m asl) was located along a dirt road inside the reserve in a secondary forest edge that included a 30 m wide natural pond and open areas. Transect B (200–400 m asl) covered forest trails and riparian environments within a mature forest. Transect C (400–1,000 m asl) comprised an old wood road (4 km) in a secondary forest and an indigenous trail (7 km) within a pristine environment that included natural ponds and riparian habitats along the trail.

From January 2012 to December 2012, Transect A or B was sampled weekly during the day (6:00–11:00 h) and at night (18:00–22:00 h), totaling 27 field days with four person hours (ph) per transect for a total search effort of 1,728 ph. Once a year, between 2012 and 2017, six expeditions at Transect C were conducted, totaling 13 field days of diurnal and nocturnal monitoring and a search effort of 1,560 ph. A leaf litter plot survey (Scott 1976) was used to sample ten plots (8 x 8 m) in Chimú peak (2014, 2017) and Plátano peak (2013, 2015) on an annual basis, for a total of 40 sampled plots.

The following information was recorded for the species detected during monitoring: 1. Holdridge altitudinal belts: basal (b) or premontane (p); 2. Type of forest: mature (M) or disturbed (D; includes secondary forest and open areas), and 3. Habitat association: riparian (R), forest (F), or swamp (S; including temporary or permanent ponds). If possible, one specimen per species was collected on each Holdridge life zone. The collected specimens were anesthetized and euthanized with lidocaine, fixed in a 10% buffered formalin solution, and later preserved in 70% ethanol solution. For all specimens, tissue samples

of muscle and liver were collected and fixed in 95% ethanol. Voucher specimens and tissue samples were deposited at the Museo de Zoología of the Universidad de Costa Rica (UCR). Some specimens were collected by third parties or other VRP researchers through occasional encounters in random field trips.

The species list includes the information obtained from this monitoring effort and UCR records of the Victoria locality, covering a study area of 51 km², hereafter referred to as Veragua. Additional photographic material from collaborations with specialists in this area was evaluated. The taxonomic nomenclature follows Frost (2019), except for hylids in which Faivovich et al. (2018) was followed. The conservation status of each species was categorized according to the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN 2019) and registered observations on the natural history of threatened species.

Data analysis and permission. The Jaccard index (I_j) was used to determine the similarity in species composition between altitudinal belts, forest types, and habitat association. A species accumulation curve was performed to account for species richness. Sampling was conducted under research permit SINAC-ACLAC-PIME-VS-R-024-2016, granted by Sistema Nacional de Areas de Conservación (National System of Conservation Areas, SINAC).

Results

Overall Results

The surveys recorded a total of 68 species of amphibians, including 64 anurans distributed in 11

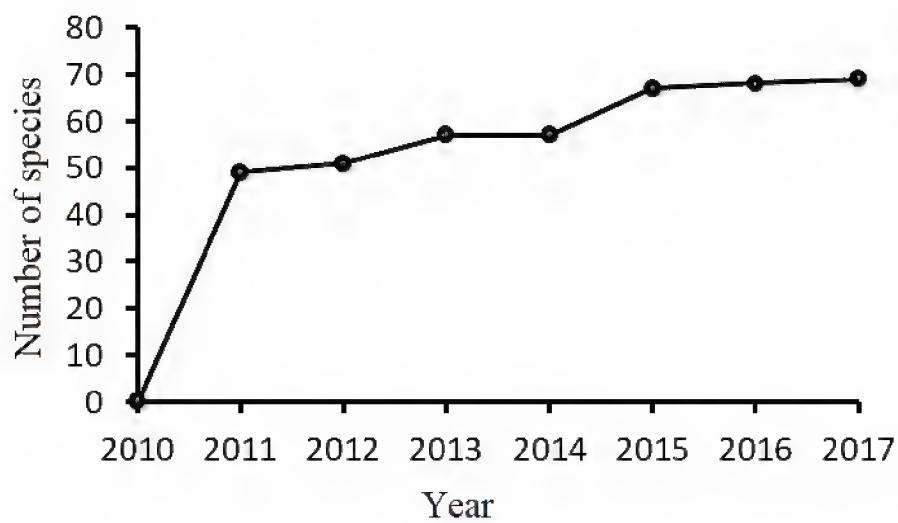


Fig. 2. Amphibian species accumulation curve for the 2010–2017 period in the study area.

families and 31 genera, three salamanders of the family Plethodontidae in two genera, and one caecilian in the family Caeciliidae (Table 1; Plates I–V). The most speciose families were Hylidae with 22 species (32.4%), followed by Craugastoridae with 15 species (21.7%) and Centrolenidae with 10 species (14.5%) [Table 1]. Six of the 68 species (8.7%) were endemic to the Atlantic slope of Costa Rica: *Bolitoglossa alvaradoi*, *Oedipina berlina*, *Craugastor persimilis*, *Diasporus amirae*, *Hyalinobatrachium diana*, and *Ecnomiohyla sukia*. The species accumulation curve reached an asymptotic phase at the end of the sampling period (Fig. 2).

Low species similarity was obtained between basal and premontane belts ($I_j = 0.37$) and the majority of premontane species were found in the basal belt, except for *C. persimilis*, *D. amirae*, and *Pristimantis caryophyllaceus* (Table 1; Fig. 3a). Mature forests and disturbed areas were found to share slightly more than half of the species ($I_j = 0.52$). A total of 19 species were only present in mature forests, and 14 species were detected only in disturbed areas (Table 1; Fig. 3b). The most diverse habitats were the forest (43 sp.) and riparian (31 sp.) environments, while 20 species were associated with swamps (Fig. 3c; Table 1). The results show that 44 species were only found in one type of habitat; out of these, the forest (19 sp.) was the most diverse habitat, followed by riparian environments (16 sp.) and swamps (9 sp.; Table 1). A medium-low similarity was found in the composition of species between the riparian and the forest (15 sp.; $I_j = 0.25$), as well as between the swamps and the forest (11 sp.; $I_j = 0.21$), although the data indicated only a minimal similarity when comparing rivers and swamps (2 sp.; $I_j = 0.04$; Table 1). The only species that were found in all three habitats were *Agalychnis spurrelli* and *Rhinella horribilis*.

According to the IUCN conservation status, one species is categorized as Data Deficient (DD), 54 as Least Concern (LC), and eight in the various threatened categories. *Pristimantis altae* and *P. caryophyllaceus* are categorized as Near Threatened (NT); *Craugastor persimilis* as Vulnerable (VU); *Duellmanohyla uranochroa*, *Ecnomiohyla veraguensis*, *Gastrotheca cornuta*, and *Bolitoglossa alvaradoi* as Endangered

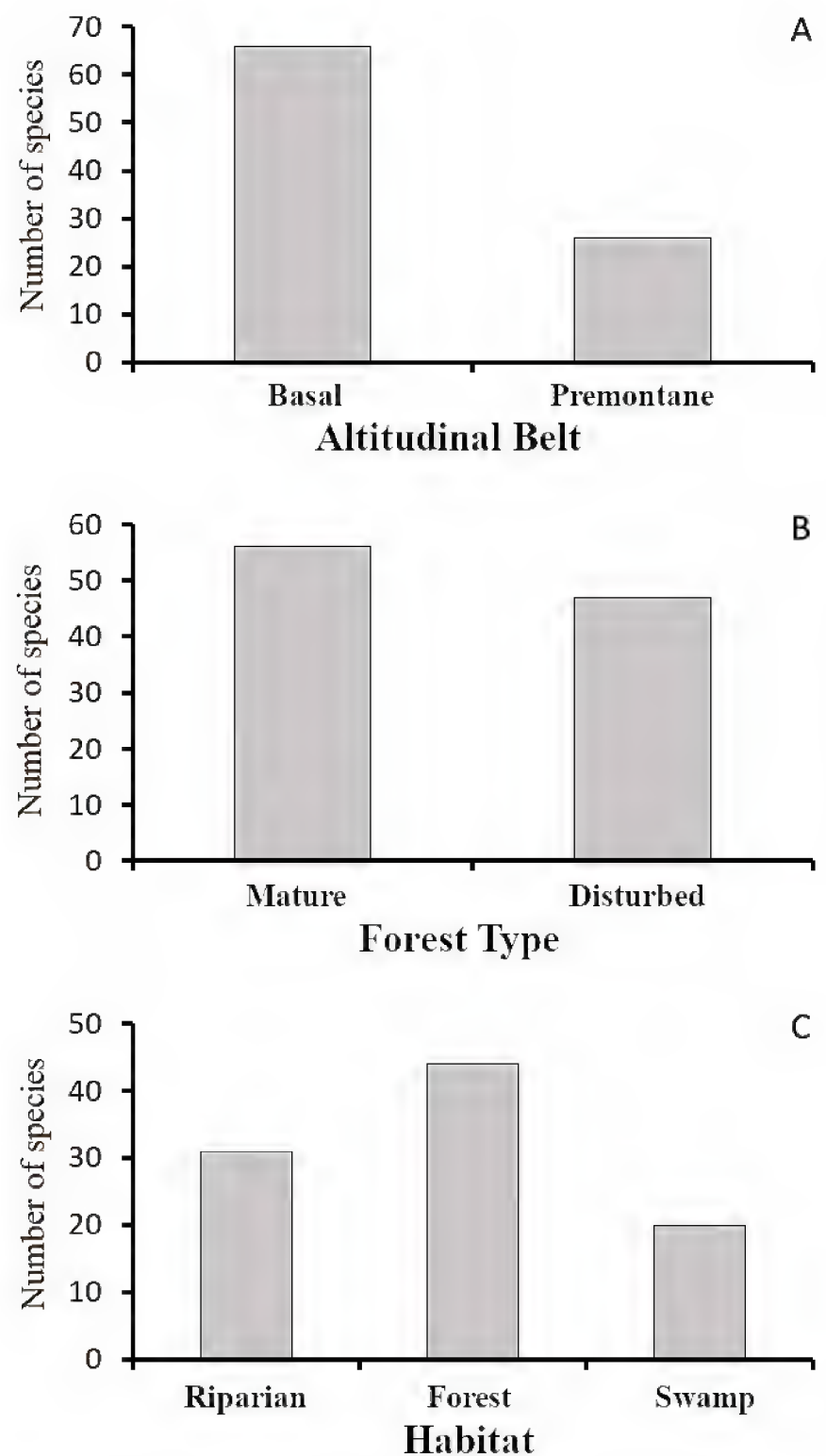


Fig. 3. Number of species registered according to the altitudinal belt (A), forest type (B), and habitat (C) in the study area.

(EN); and *Agalychnis lemur* as Critically Endangered (CR; Table 1). The species *Hyalinobatrachium diana*, *D. amirae*, *C. sylviae*, *E. sukia*, *Ecnomiohyla bailarina*, and *O. berlina* remain uncategorized (Table 1).

Regarding the uncategorized species populations, few populations of *H. diana* were observed in isolated streams within the basal mature forest. Populations with several individuals of *Diasporus amirae* were detected at the premontane belt. The species *C. sylviae* was found during 2011–2012 in only a few places within the forest. Generally, the adults were observed inside tree holes up to 3 m high. However, between 2012 and 2017, the species was more commonly observed reproducing throughout the year in VRP, near small artificial ponds (length 200 cm, width 150 cm, depth 50 cm) located within the forest. These ponds were created in 2012 by a project of the Veragua Foundation for the Rainforest Research “Veragua Foundation” (NGO) that aims to establish *in situ* breeding sites for the conservancy and study of the native amphibians. *Ecnomiohyla bailarina* and *E. sukia* were detected calling from the canopy in basal and premontane pristine forest. *Oedipina berlina*

Extreme frog diversity in Costa Rica

Table 1. Checklist of amphibians of the Veragua Rainforest Eco Research and Adventure Park and its surroundings, with information on the voucher ID (UCR), IUCN status, altitudinal belt (basal [b] / premontane [p]), forest type (mature [M] / disturbed [D]), and habitat association (forest [F] / swamp [S] / riparian [R]).

Taxa	UCR	IUCN status	Altitudinal Belts	Forest Types	Habitats
Aromobatidae (1)					
<i>Allobates talamancae</i> (Cope, 1875)	21593	LC	b p	M D	F S
Bufonidae (4)					
<i>Incilius coniferus</i> (Cope, 1862)	21164	LC	b	M D	F S
<i>Incilius melanochlorus</i> (Cope, 1877)	21983	LC	b p	M	R F
<i>Rhaebo haematiticus</i> Cope, 1862	21139	LC	b p	M D	R F
<i>Rhinella horribilis</i> (Wiegmann, 1833)	21148	LC	b	D	R F S
Centrolenidae (10)					
<i>Cochranella granulosa</i> (Taylor, 1949)	23183	LC	b	M D	R
<i>Espadarana prosoblepon</i> (Boettger, 1892)		LC	b	D	R
<i>Hyalinobatrachium chirripoi</i> (Taylor, 1958)	21431	LC	b p	M D	R
<i>Hyalinobatrachium diana</i> e Kubicki, Salazar, and Puschendorf, 2015	22035		b	M	R
<i>Hyalinobatrachium fleischmanni</i> (Boettger, 1893)	23182	LC	b	M D	R
<i>Hyalinobatrachium talamancae</i> (Taylor, 1952)	21157	LC	b p	M	R
<i>Hyalinobatrachium valerioi</i> (Dunn, 1931)	21140	LC	b	M	R
<i>Sachatamia albomaculata</i> (Taylor, 1949)	21114	LC	b p	M D	R
<i>Teratohyla pulverata</i> (Peters, 1873)	21153	LC	b	M D	R
<i>Teratohyla spinosa</i> (Taylor, 1949)	21126	LC	b	M D	R
Craugastoridae (15)					
<i>Craugastor brandsfordi</i> (Cope, “1885,” 1886)	21149	LC	b	D	F
<i>Craugastor crassidigitus</i> (Taylor, 1952)	21120	LC	b p	M D	R F
<i>Craugastor fitzingeri</i> (Schmidt, 1857)	21150	LC	b p	M D	F
<i>Craugastor gollmeri</i> (Peters, 1863)	22550	LC	b p	M	F
<i>Craugastor megacephalus</i> (Cope, 1875)		LC	b	M	F
<i>Craugastor mimus</i> (Taylor, 1955)	21414	LC	b	M D	F
<i>Craugastor noblei</i> (Barbour and Dunn, 1921)	21156	LC	b	M D	F
<i>Craugastor persimilis</i> (Barbour, 1926)	22529	VU	b	M	F
<i>Craugastor polyptychus</i> (Cope, 1886)	21121	LC	b p	M D	F
<i>Craugastor talamancae</i> (Dunn, 1931)		LC	b	D	R F
<i>Pristimantis altae</i> (Dunn, 1942)	21145	NT	b p	M D	R F
<i>Pristimantis caryophyllaceus</i> (Barbour, 1928)	21844	NT	p	M	F
<i>Pristimantis cerasinus</i> (Cope, 1875)	21127	LC	b p	M D	F
<i>Pristimantis cruentus</i> (Peters, 1873)	21170	LC	b p	M	R F
<i>Pristimantis ridens</i> (Cope, 1866)	21096	LC	b p	M D	R F
Dendrobatidae (4)					
<i>Dendrobates auratus</i> (Girard, 1855)	21128	LC	b	M D	F
<i>Oophaga pumilio</i> (Schmidt, 1857)	21106	LC	b p	M D	R F
<i>Phyllobates lugubris</i> (Schmidt, 1857)	21143	LC	b	M D	R F
<i>Silverstoneia flotator</i> (Dunn, 1931)	21986	LC	b p	M D	R F
Eleutherodactylidae (2)					
<i>Diasporus diastema</i> (Cope, 1875)	21415	LC	b p	M D	R F
<i>Diasporus amirae</i> Arias, Chaves, Salazar, Salazar-Zúñiga, and García-Rodríguez, 2019	22010		p	M	F

Table 1 (continued). Checklist of amphibians of the Veragua Rainforest Eco Research and Adventure Park and its surroundings, with information on the voucher ID (UCR), IUCN status, altitudinal belt (basal [b] / premontane [p]), forest type (mature [M] / disturbed [D]), and habitat association (forest [F] / swamp [S] / riparian [R]).

Taxa	UCR	IUCN status	Altitudinal Belts	Forest Types	Habitats
Hemiphractidae (1)					
<i>Gastrotheca cornuta</i> (Boulenger, 1898)	21017	EN	b p	M	R
Hylidae (23)					
<i>Agalychnis callidryas</i> (Cope, 1862)	21098	LC	b p	M D	F S
<i>Agalychnis lemur</i> (Boulenger, 1882)	21104	CR	b p	M D	S
<i>Agalychnis saltator</i> Taylor, 1955	21429	LC	b	M D	F S
<i>Agalychnis spurrelli</i> (Boulenger, 1913)	21119	LC	b	M D	R F S
<i>Boana rufitela</i> (Fouquette, 1961)		LC	b	M	S
<i>Cruziohyla sylviae</i> Gray, 2018	21422		b	M D	F S
<i>Dendropsophus ebraccatus</i> (Cope, 1886)	21103	LC	b	M D	S
<i>Dendropsophus phlebodes</i> (Stejneger, 1906)	21432	LC	b	D	S
<i>Duellmanohyla rufiocularis</i> (Taylor, 1952)		LC	b p	M	R
<i>Duellmanohyla uranochroa</i> (Cope, 1875)	22002	EN	b p	M	R F
<i>Ecnomiohyla bailarina</i> Batista, Hertz, Mebert, Köhler, Lotzkat, Ponce, and Vesely, 2014	22287		b	M	F
<i>Ecnomiohyla sukia</i> Savage and Kubicki, 2010	22940		b p	M	F
<i>Ecnomiohyla veraguensis</i> Batista, Hertz, Mebert, Köhler, Lotzkat, Ponce, and Vesely, 2014	21941	EN	b p	M	F
<i>Hyloscirtus palmeri</i> (Boulenger, 1908)	21995	LC	b	M D	R
<i>Isthmohyla lancasteri</i> (Barbour, 1928)	21994	LC	b	M	R
<i>Scinax boulengeri</i> (Cope, 1887)		LC	b	D	S
<i>Scinax elaeochroa</i> (Cope, 1875)	21151	LC	b	D	F S
<i>Smilisca manisorum</i> (Taylor, 1954)		LC	b	D	S
<i>Smilisca phaeota</i> (Cope, 1862)	21113	LC	b	M D	S
<i>Smilisca puma</i> (Cope, 1885)		LC	b	D	S
<i>Smilisca sordida</i> (Peters, 1863)	21099	LC	b	M D	R
<i>Tlalocohyla loquax</i> (Gauge and Stuart, 1934)	21097	LC	b	M D	S
Leptodactylidae (2)					
<i>Leptodactylus melanonotus</i> (Hallowell, 1861)	21518	LC	b	D	F S
<i>Leptodactylus savagei</i> Heyer, 2005	20107	LC	b	M D	F S
Microhylidae (1)					
<i>Hypopachus pictiventris</i> (Cope, 1886)		LC	b	D	S F
Ranidae (2)					
<i>Lithobates vaillanti</i> (Brocchi, 1877)	21101	LC	b	D	R
<i>Lithobates warszewitschii</i> (Schmidt, 1857)	21102	LC	b	M D	R F
Plethodontidae (3)					
<i>Bolitoglossa alvaradoi</i> Taylor, 1952	22048	EN	b	M	F
<i>Bolitoglossa colonnea</i> (Dunn, 1924)	21178	LC	b p	M D	F
<i>Oedipina berlini</i> Kubicki, 2016	22882		b	D	F
Caeciliidae (1)					
<i>Caecilia volcani</i> Taylor, 1969		DD	b	D	F



Plate I. Photos in life of amphibians recorded in the sampling area: (A) *Allobates talamancae*; (B) *Incilius coniferus*; (C) *I. melanochlorus*; (D) *Rhaebo haematiticus*; (E) *Rhinella horribilis*; (F) *Cochranella granulosa*; (G) *Espadarana prosoblepon*; (H) *Hyalinobatrachium chirripoi*; (I) *H. diana*; (J) *H. fleischmanni*; (K) *H. talamanca*; (L) *H. valerioi*; (M) *Sachatamia albomaculata*; (N) *Teratohyla pulverata*; and (O) *T. spinosa*. Photos by Victor Acosta-Chaves (C, G, N); Javier Lobón-Rovira (A, F, L, M, O); José Andrés Salazar-Zúñiga (B, D, E, H, J, K); Andréi Solís (I).

was observed once in the secondary basal forest, as an individual was on the leaf litter on one of the trails of the Veragua Rainforest Park at night.

Observations on the Threatened Species

Pristimantis altae (NT; Plate II-K) was seen and heard in mature and secondary forests (Table 1). At the basal belt,

isolated males were recorded on riversides and inside the forest; nevertheless, at the premontane belt, groups of at least six calling males were registered, found very close to each other (2–3 m apart). At the premontane belt, *P. caryophyllaceus* (NT; Plate II-L) was commonly observed perched on leaves in the understory (100–150 cm high). On one occasion, a female was found in brooding position over a fully developed clutch with 26



Plate II. Photos in life of amphibians recorded in the sampling area: (A) *Craugastor brandsfordi*; (B) *C. crassidigitus*; (C) *C. fitzingeri*; (D) *C. gollmeri*; (E) *C. megacephalus*; (F) *C. mimus*; (G) *C. noblei*; (H) *C. persimilis*; (I) *C. polyptychus*; (J) *C. talamancae*; (K) *Pristimantis altae*; (L) *P. caryophyllaceus*; (M) *P. cerasinus*; (N) *P. cruentus*; (O) *P. ridens*. Photos by Víctor Acosta-Chaves (C, F); Javier Lobón-Rovira (B, K, O); José Andrés Salazar-Zúñiga (A, D, E, G–J, L–N).

eggs inside a partially rolled leaf at 1 m high. The female and her eggs were collected and placed in a plastic bag, and the next day all the eggs had hatched inside the bag. *Craugastor persimilis* (VU; Plate II-H) was observed several times during the plot survey, hidden in the leaf litter in the premontane belt.

Duellmanohyla uranochroa (EN; Plate IV-E) was detected in a few streams in pristine forest (Table 1). Males were observed calling from the streamside

vegetation. Also, a male chorus was heard inside the forest at a distance of at least 100 m from the nearest stream. Some of these individuals were located in between the aerial roots of a walking palm (*Socratea exorrhiza*). *Ecnomiohyla veraguensis* (EN; Plate IV-H) was observed once calling from the canopy in the basal mature forest. *Gastrotheca cornuta* (EN; Plate III-G) was uncommon in the survey samplings. Only two populations are known in the study area; one of them was last reported in 1996



Plate III. Photos in life of amphibians recorded in the sampling area (unless otherwise specified, all photographs refer to specimens detected in Veragua): (A) *Dendrobates auratus*; (B) *Oophaga pumilio*; (C) *Phyllobates lugubris*; (D) *Silverstoneia flotator*; (E) *Diasporus diastema*; (F) *Diasporus amirae*; (G) *Gastrotheca cornuta* (Veraguas, Panama); (H) *Agalychnis callidryas*; (I) *A. lemur*; (J) *A. saltator*; (K) *A. spurrelli*; (L) *Boana rufitela*. Photos by Abel Batista (G); Javier Lobón-Rovira (A–C, E, H, I, K, L); José Andrés Salazar-Zúñiga (D, F, J).

at 200 m asl in the Victoria river basin (Solorzano et al. 1998), and the subsequent record was published 16 years later, in the streamside vegetation in a deep canyon of the Zent River at 550 m asl (Salazar 2015).

Three populations of the Critically Endangered *Agalychnis lemur* (Plate III-I) were observed throughout Veragua. One of the populations was located in a pond (width 35 m) in a mature premontane forest, where many males were observed calling from the vegetation at 50–150 cm high. The other two populations were found in secondary forest at the basal belt, including a population that is on the border of the VRP (Table 1). Outside the reserve, this species was observed in small ponds and flooding banks next to a wood extraction road. Thus, given this immediate threat, small artificial ponds (length 200 cm, width 150 cm, depth 50 cm) were created by the Veragua Foundation during 2015

to protect this population. As of 2016, it was easier to observe individuals throughout the year during high-humidity nights near these reproductive sites.

Discussion

With 215 species, Costa Rica is the 19th richest country in the world for amphibians, and exhibits the highest richness per unit area in Middle America (Kubicki 2008; Frost 2019). In this region, amphibian species density seems to be greater towards the South, specifically in Costa Rica and Panama (Campbell 1999). The results presented here show that Veragua exhibits the highest known species richness among Middle American lowland evergreen forests. With a notable anuran representation of 64 species in 51 km² surveyed (Table 1; Plates I–V), these



Plate IV. Photos in life of amphibians recorded in the sampling area (unless otherwise specified, all photographs refer to specimens detected in Veragua): (A) *Cruziohyla sylviae*; (B) *Dendropsophus ebraccatus*; (C) *D. phlebodes*; (D) *Duellmanohyla rufiocularis*; (E) *D. uranochroa*; (F) *Ecnomihyla bailarina* (Darien, Panama); (G) *E. sukia*; (H) *E. veraguensis* (Veraguas, Panama); (I) *Hyloscirtus palmeri*; (J) *Isthmohyla lancasteri*; (K) *Scinax boulengeri*; (L) *S. elaeochroa*; (M) *Smilisca manisorum*; (N) *S. phaeota*; (O) *S. puma*; (P) *S. sordida*; (Q) *Tlalocohyla loquax*. Photos by Víctor Acosta-Chaves (M); Abel Batista (F); Edwin Gómez-Méndez (O); Daniel Hernández (C); Andreas Hertz (H); Javier Lobón-Rovira (A, B, J, N); José Andrés Salazar-Zúñiga (D, E, G, I, K, L, P, Q).



Plate V. Photos in life of amphibians recorded in the sampling area: (A) *Leptodactylus melanonotus*; (B) *L. savagei*; (C) *Hypopachus pictiventris*; (D) *Lithobates vaillanti*; (E) *L. warszewitschii*; (F) *Bolitoglossa alvaradoi*; (G) *B. colonnea*; (H) *Oedipina berlina*; (I) *Caecilia volceni*. Photos by Víctor Acosta-Chaves (C, D, F); Esmeralda Arévalo (I); Javier Lobón-Rovira (E); José Andrés Salazar-Zúñiga (A, B, G, H).

surveys reveal one of the highest numbers of amphibian species reported per unit area in the Neotropics (Savage 2002; Boza-Oviedo et al. 2012; Barrio-Amorós et al. 2011; Hertz et al. 2012; Arias and Bolaños 2014; Ferreira et al. 2017).

In comparison with the most important diversity hot spots from South America, the richest region of amphibian species worldwide (Ron et al. 2018; Frost 2019), Veragua is also one of the most diverse localities in the Neotropics with 68 species. Only certain sites across the Amazon lowlands exhibit a greater richness of amphibians than these Veragua sites (Barrio-Amorós et al. 2011; Ferreira et al. 2017). For example, amphibian richness in Brazil ranged from 18 species (Alter do Chão, Pará) to as many as 78 species along a small section of the Juruá river (Zimmermann and Rodrigues 1990; Lima 2008; Queiroz et al. 2011; Pereira-Júnior et al. 2013; Araújo and Costa-Campos 2014; Alves-Binicio and Dias-Lima 2017; Ferreira et al. 2017; Lima et al. 2017), with the extreme exception of 109 amphibian species in the middle of the Xingu River (Vaz-Silvia et al. 2015; Ferreira et al. 2017). In Peru, the most diverse sites are in Bajo Río Lullapichis (74 sp.; Schluter et al. 2004), Parque Nacional Manu (68 sp.; Morales and McDiarmid 1996), and Cuzco Amazónico (64 sp.; Duellman 2005; Barrio-Amorós et al. 2011). In Colombia, the highest diversity was found in Leticia 97 species (Lynch 2005),

and the two most important inventories reported in Ecuador are from the village of Santa Cecilia (87 sp.; Duellman 1978) and Parque Nacional Yasuní (135 sp.), currently the most diverse amphibian site in the world (Ron et al. 2018).

In Costa Rica, the amphibian richness is concentrated in the southern lowlands of the country and in the northeastern Atlantic versant (Campbell 1999; McDiarmid and Savage 2005; Santos-Barrera et al. 2008). Compared to the three other major inventories in these areas, Veragua is more species-rich than either the South Pacific locality of Rincón (47 sp., Anura [42]/Caudata [4]/Gymnophiona [1]; McDiarmid and Savage 2005), the Atlantic versant sites of La Selva Biological Station (52 sp., 47/3/1; Guyer and Donnelly 2005), or Guayacán (66 sp., 58/6/2; Kubicki 2008). Other important inventories were reported in a transitional wet-dry forest in the locality of Carara (39 sp.; Laurencio and Malone 2009), and among the richest sites in dry forests is Finca Taboba, at the northern edge of the country (21 sp.; Campbell 1999).

The low diversity of salamanders found in Veragua could be due to fact that the sampling area was below 1,100 m asl, and that only one monitoring was conducted per year in the premontane belt. It is also possible that the sampling method was not inclusive enough to cover a broader diversity in the basal belt. The number of frog and

salamander species distributed along specific elevations in Middle America follows a pattern previously noted in Guatemala and Belize, i.e., a moderate number of species in the lowlands that progressively increases as it reaches moderate or intermediate elevations, and then declines precipitously at higher elevations (Campbell and Vannini 1989; Wells 2007). Salamanders partly show this pattern as they exhibit a more dramatic increase in species in the highlands (Campbell 1999; Wells 2007). We presume that some other species, such as *Bolitoglossa striatula*, *Nototriton matama*, *Oedipina carablanca*, and *Oedipina gracilis*, which occur in the Atlantic Versant at a similar altitudinal belt and close to the Talamanca mountain range (Savage 2002; Kubicki 2008; Leenders 2016), could also occur in Veragua. Among caecilids, only *Caecilia volceni* was found. The apparent low diversity in this group is probably caused by its fossorial habits (Peloso 2010; Ferreira et al. 2017) which undermine effective sampling. We think that at least one common caecilid species in the Atlantic Versant (*Gymnophis multiplicata*) could be present in Veragua (Leenders 2016).

In this study, the basal belt was found to be more diverse than the premontane (Fig. 3; Table 1). This is a generalized pattern of distribution among anurans (Savage 2002) and should account for the vast representation of frogs and toads in this study, which represent 94% of the total amphibian species. The latter species distribution is similar to that of the wet slopes of the Andes, the region with the highest diversity of anuran species in the world (Duellman 1999b; Wells 2007). The higher number of anuran species in old mature forests compared with disturbed areas might be explained by the presence of more microhabitats in pristine environments (Table 1; Savage 2002; Acosta-Chaves et al. 2015). Nevertheless, one of the main factors shown to influence high local and regional diversity is the variety of habitats with numerous vegetation types, ranging from forests to open grasslands, that occur side-by-side in the landscape, each of them harboring a different array of species (Colli et al. 2002; Nogueira et al. 2009; Lopes-Santos et al. 2014).

The greatest species diversity and the highest level of endemism for amphibians in Middle America occur along the windward mesic slopes of major mountain ranges between elevations of 800 and 2,800 m asl, which in Costa Rica include the Guanacaste, Tilarán, and Talamanca mountain ranges (Campbell 1999). The Talamanca mountain range is recognized as a site of speciation and a dispersion center for several species with a high degree of endemism (Arias and Bolaños 2014). Among amphibians and reptiles, 27% of the species in Costa Rica are endemic to this region (Campbell 1999; Savage 2002; Chaves et al. 2009; Streicher et al. 2009; Boza-Oviedo et al. 2012; Arias and Bolaños 2014). In this study, 8.7% of the amphibians are endemic to the Atlantic Versant of Costa Rica (Campbell 1999; Leenders 2016; Frost 2019), including species of the genera *Duellmanohyla* and *Isthmohyla*, which are endemic to

Middle America (Faivovich et al. 2018).

A high number of species was found along the forest and the riparian habitats (Fig. 3; Table 1). This association with a type of forest might be due to the great variety of microhabitats found throughout these environments that result from irregular topography (Wells 2007; Kubicki 2008). Leaf litter is an important habitat for anurans, especially among species with terrestrial reproduction (although it is not restricted to them; Wells 2007). The high moisture levels found in the forest floor allow terrestrial species to forage and call during either the day or night (Wells 2007). The species richness of leaf litter frogs and toads is positively correlated with the number of wet months and the litter mass depth (Wells 2007; Whitfield et al. 2007).

The distribution along the riparian habitats was found to be non-uniform, as it could rely on vegetation coverage and the physical characteristics of the environment. The high number of amphibian species found along riparian habitats in this study could be due to the numerous springs, streams, and torrents found throughout the sampling area (Fig. 1), which generate several microhabitats along this environment (McDiarmid and Savage 2005; Kubicki 2007; SINAC 2018). Previous studies found a similar distribution pattern in Guayacán, where 35% of the species are associated with lotic environments (Kubicki 2008), while glass frogs represent more than 16% of the amphibian diversity in La Selva (Guyer and Donnelly 2005) and Rincón (McDiarmid and Savage 2005). In the mountainous regions of Middle America, the permanent or temporary ponds required for amphibian breeding are often scarce (Wells 2007). This pattern is also seen at Veragua and Guayacán, where the highly irregular topography of these localities causes permanent ponds to be a much more limited resource for reproduction (Kubicki 2008). Nevertheless, pond-breeders account for a notable representation of anuran species in Veragua (32, 4%) and Guayacán (30%; Kubicki 2008).

The species accumulation curve reached an asymptote, meaning that the sampling effort to detect species produced a number near the maximum expected value (Fig. 2). Nevertheless, there may be some cryptic species groups with high variation, and this fact may obscure the estimates given here, as different species could be hidden under a single name (Funk et al. 2012; Alves-Binicio and Dias-Lima 2017). However, the clarification of such unknown diversity requires further integrative taxonomic studies. Likewise, we suggest a larger survey effort in the premontane belt, since the difficult access to sampling areas did not allow for a continuous sampling.

Comments on Threatened Species

This study registered *P. altae* (NT), which has been previously reported in very few places on the Atlantic Slope of Costa Rica and northwestern Panama (Leenders 2016). Overall, there is only limited information about

its natural history, population trends, and conservation needs (Leenders 2016). Historically, *P. altae* has been associated with undisturbed areas (Savage 2002; Pounds et al. 2008a). Even though this species was observed in mature forests, it was also commonly detected close to streams within secondary forests. According to Savage (2002), this species is mute; however, during high humidity dark nights it is common to hear the species emitting a short two note call “clock-clock,” similar to the sound that two glass marbles emit when they hit each other twice very rapidly. In basal secondary forests, isolated males were heard calling in the forest. However, on the premontane belt, this species is more abundant, and several males were heard calling at a close distance from each other. Unfortunately, the calls are emitted sporadically, and have not yet been recorded.

In this study, *P. caryophyllaceus* (NT) was one of the most common frogs in the mature premontane belt. During the mid-1980s, when many populations in Costa Rica declined (Leenders 2016), *P. caryophyllaceus* disappeared from most lowlands; however, it persisted at higher elevations (Leenders 2016). This pattern is rare among Neotropical anurans, considering that more pronounced declines generally occur at mid- and high elevations; in Panama, populations declined dramatically in some highlands, but in Costa Rica they seem to be recovering in areas above 800 m asl (Savage 2002; Pounds et al. 2008b; Leenders 2016). A female was found inside a rolled leaf at 100 cm above the forest floor attending a mass of 29 eggs. This same behavior was previously reported in Panamanian populations by Myers (1969). Recent research showed that *Craugastor persimilis* (VU) is susceptible to habitat fragmentation and it is often absent in open pasture lands and pineapple plantations (Bolaños et al. 2008). This observation is consistent with the observations reported here, as this species was only observed in the mature premontane forest.

Duellmanohyla uranochroa (EN) was a historically common species across humid lowland and mountain forests (Savage 2002). However, it has declined precipitously since the late 1980s. By 2002, *D. uranochroa* had experienced a significant decline across several populations in Costa Rica (Duellman 2001; Savage 2002; Leenders 2016; IUCN 2019). However, since 2007 some populations have reappeared in Monteverde, the Matama mountains, and Tuis de Turrialba (NatureServe and IUCN 2013), as well as in western Panama (Hertz et al. 2012). In this monitoring effort, populations were observed at the premontane and basal belt of the mature forest, sometimes close to riparian environments. Generally, males were found on the forest floor or on walking palm roots, and up to 2 m high. Similar behaviors have been reported in other populations of this species (Duellman 2001; Savage 2002; NatureServe and IUCN 2013).

Before this report, *Ecnomiohyla veraguensis* was only known from two small Panamanian populations in Santa Fé National Park, where it is highly threatened by

ongoing habitat modification due to forest clearance for agriculture and open pit mining (IUCN SSC Amphibian Specialist Group 2019). *Ecnomiohyla veraguensis* is differentiated here from *E. miliaria*, another congener from the Caribbean foothills, based on the presence of scalloped fleshy fringes and the absence of heel tubercles in the former (Batista et al. 2014); from *E. bailarina*, considering that *E. veraguensis* has a finely tuberculate dorsum (strongly tuberculate in *E. bailarina*) with scattered minute keratin tipped tubercles on the posterior part of the body and 6–8 widely spaced, keratinized black spines bordering the outer side of the thumb (two clusters of numerous, small nuptial spines in *E. bailarina*; Batista et al. 2014). The most similar species to *E. veraguensis* is *E. sukia*; however, the latter lacks nuptial spines in adult males (Batista et al. 2014).

Gastrotheca cornuta (EN) is considered a rare species in Colombia and Costa Rica, while it has declined in Ecuador and Panama (Coloma et al. 2008; AmphibiaWeb 2009). In Costa Rica, this species is known from only three localities in the Limón Province (Coloma et al. 2008). The first specimen was collected during 1984 in the northwest of Nimaso peak in the Talamanca Mountain range at 700 m asl (Solorzano et al. 1998; Savage 2002). The other two localities were reported in Veragua, also at basal mature forests (Solorzano et al. 1998; Salazar 2015).

Bolitoglossa alvaradoi (EN) was only observed once in the mature forest. This endemic species has only been reported in undisturbed areas and it is considered endangered because its extent of occurrence is less than 5,000 km² (Bolaños et al. 2008). This salamander is a rare species, mostly due to its secretive arboreal habitats (Bolaños et al. 2008). In the current survey, this species was found during the day near a small stream on a leaf at 100 cm. Some other studies reported individuals inside bromeliads and leaf axils during the day (Wake 1987; Savage 2002).

Agalychnis lemur (CR) occurs in Costa Rica, Panama, and marginally in Colombia (Solís et al. 2008). It inhabits basal and premontane humid forests and has been historically associated with pristine areas (Duellman 2001; Savage 2002). This species has always been fairly uncommon throughout its range; however, it was listed as Critically Endangered because of ongoing drastic population declines, estimated to be more than 80% over a ten-year period (Solís et al. 2008). This survey found three separate natural breeding populations in the study area. One of these populations was already reported in Costa Rica and was considered the only remnant wild breeding population (Solís et al. 2008). Another small population was reported in Guayacán (Kubicki 2008; Solís et al. 2008). All other previously known Costa Rican populations of this species have disappeared, including those in Monteverde, San Ramón, Braulio Carrillo, and Tapantí (Solís et al. 2008).

The main threats reported for *A. lemur* are habitat

destruction and chytridiomycosis (Solís et al. 2008). In one of the reported populations in this study, Whitfield et al. (2017) found a low infection prevalence (<10%, $n = 20$) of *Batrachochytrium dendrobatidis* (*Bd*) and a low infection intensity among infected individuals. Some studies demonstrated that highly *Bd*-susceptible amphibians persist in environments hostile to *Bd*, even when *Bd* is still present (Puschendorf et al. 2011). The samplings reported here never registered a sick animal. Nonetheless, in some places where it was common to see the species during the sampling surveys, *A. lemur* had disappeared after the intensification of wood extraction during 2013. *Agalychnis lemur* appears to be highly susceptible to habitat loss, and the lack of natural reproductive sites in the forest promotes the use of the flooding banks or small ponds at the forest edge (JASZ, pers. obs.), a condition that we consider makes this species extremely vulnerable to habitat fragmentation.

This study shows that the threatened species reported here are associated with mature forest. These species may be sensitive to changes in their environment and might therefore exhibit a low tolerance to human impact (Dixo and Martins 2008; Lopes-Santos et al. 2018). The main biodiversity threats observed while conducting this study were: 1. Habitat destruction (legal or illegal) due to population growth, pastures, and extraction labors for wood and stone; 2. Monocultures of extensive plantations (e.g., by banana and pineapple corporations in the nearby lowlands), that also create substantial soil erosion and use numerous agrochemicals to maintain the crops, producing substantial amounts of pollution residues (Castillo et al. 1997; Castillo and Ruepert 2001; Sasa et al. 2010); 3. Illegal wildlife extraction; 4. Little control by the responsible authorities; and 5. Low levels of environmental education.

Conclusions

This survey shows that Veragua is a high priority conservation area with 11.7% of its amphibian diversity under the IUCN threatened categories, out of which five species are cataloged as Endangered or Critically Endangered (Table 1; IUCN 2019). In addition, this study reports *E. veraguensis* in Costa Rica for the first time and represents the only locality known for *E. bairdina* (Kubicki and Salazar 2015). The diversity analysis reveals one of the most important amphibian hot spots in the Neotropics, with evidence of recent sightings of several species after concerning declines (like those of *Duellmanohyla uranochroa* and *Agalychnis lemur*), and contrasts with the decimated diversity in several other important locations in Costa Rica (e.g., La Selva, Rincón de Osa, Cerro Chompipe, Monteverde, Cerro de la Muerte, Tapanti, Volcán Cacao, Palmar Norte, and Las Tablas) that have declined or disappeared since the late 1980s (Whitfield et al. 2007; Sasa et al. 2010; Ryan et al. 2015). Based on these findings, we suggest a long-term

monitoring of the biodiversity in order to have control over population fluctuations, and we highly recommend natural history and behavioral studies to improve conservation actions across this biodiversity hot spot. According to the international conservation agreements, as well as Costa Rica's laws and executive decrees, the information provided in this article should help to protect the area from invasive activities that may negatively affect the biodiversity or major river basins (Sasa et al. 2010).

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Literature Cited

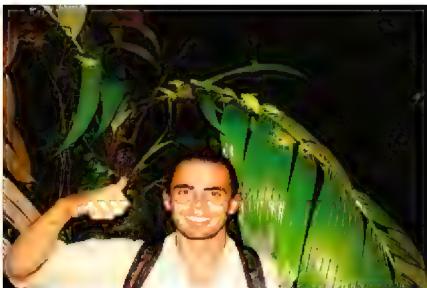
- Abarca JG. 2012. Cambios en la estructura de la comunidad de anuros (Amphibia: Anura) en el Cerro Chompipe. *Cuadernos de Investigación UNED* 4: 9–15.
- Acosta-Chaves VJ, Chaves G, Abarca JG, García-Rodríguez A, Bolaños F. 2015. A checklist of the

- amphibians and reptiles of Río Macho Biological Station, Provincia de Cartago, Costa Rica. *Check List* 11: 1–10.
- Alves-Binicio R, Dias-Lima J. 2017. Anurans of Amapá National Forest, Eastern Amazonia, Brazil. *Herpetology Notes* 10: 627–633.
- AmphibiaWeb. 2009. *Gastrotheca cornuta*. University of California, Berkeley, California, USA. Available: <http://amphibiaweb.org/species/1371> [Accessed: 1 November 2018].
- Araújo AS, Costa-Campos CE. 2014. Anurans of the Reserva Biológica do Parazinho, Municipality of Macapá, state of Amapá, eastern Amazon. *Check List* 10: 1,414–1,419.
- Arias E, Bolaños F. 2014. A checklist of the amphibians and reptiles of San Isidro de Dota, Reserva Forestal Los Santos, Costa Rica. *Check List* 10: 870–877.
- Barrio-Amorós CL, Brewer-Carías C, Fuentes O. 2011. Aproximación preliminar a la herpetocenosis de un bosque pluvial en la sección occidental de la Sierra de Lema, Guayana Venezolana. *Revista de Ecología Latinoamericana* 16: 1–46.
- Batista A, Herts A, Mebert K, Köhler G, Lotzkat S, Ponce M, Vesely M. 2014. Two new fringe-limbed frogs of the genus *Ecnomiohyla* (Anura: Hylidae) from Panama. *Zootaxa* 3826(3): 449–474.
- Becker CG, Fonseca CR, Haddad CFB, Batista RF, Prado PI. 2007. Habitat split and the global decline of amphibians. *Science* 318: 1,775–1,777.
- Bolaños F, Savage JM, Wake DB. 2008. *Bolitoglossa alvaradoi*. *The IUCN Red List of Threatened Species* 2008: e.T59138A11888362.
- Bolaños F, Savage JM, Chaves G. 2011. *Anfibios y Reptiles de Costa Rica*. Listas Zoológicas Actualizadas Universidad de Costa Rica, Museo de Zoología UCR, San Pedro, Costa Rica. Available: <http://museo.biologia.ucr.ac.cr/Listas/LZAPublicaciones.htm> [Accessed: 1 August 2018].
- Boza-Oviedo E, Rovito SM, Chaves G, García-Rodríguez A, Artavia LG, Bolaños F, Wake DB. 2012. Salamanders from the eastern Cordillera de Talamanca, Costa Rica, with descriptions of five new species (Plethodontidae: *Bolitoglossa*, *Nototriton*, and *Oedipina*) and natural history notes from recent expeditions. *Zootaxa* 3309: 36–61.
- Campbell JA. 1999. Distribution patterns of amphibians in Middle America. Pp. 111–210 In: *Patterns of Distribution of Amphibians. A Global Perspective*. Editor, Duellman WE. Johns Hopkins University Press, Baltimore, Maryland, USA. 648 p.
- Campbell JA, Vannini JP. 1989. Distribution of amphibians and reptiles in Guatemala and Belize. *Proceedings of the Western Foundation of Vertebrate Zoology* 4: 1–21.
- Castillo LE, Ruepert C. 2001. *Estudio preliminar de la calidad del agua superficial en la zona de Volcán de Puntarenas, Buenos Aires de Puntarenas*. Instituto Regional de Sustancias Tóxicas, Universidad Nacional, Heredia, Costa Rica. Available: https://www.researchgate.net/publication/326929351_ESTUDIO_PRELIMINAR_DE_LA_CALIDAD_DEL_AGUA_SUPERFICIAL_EN_LA_ZONA_DE_VOLCAN_BUENOS_AIRES_DE_PUNTARENAS [Accessed: 1 February 2019].
- Castillo LE, De La Cruz E, Ruepert C. 1997. Ecotoxicology and pesticides in tropical aquatic ecosystems of Central America. *Environmental Toxicology and Chemistry* 16: 41–51.
- Chaves G, García-Rodríguez A, Mora A, Leal A. 2009. A new species of dink frog (Anura: Eleutherodactylidae: *Diasporus*) from Cordillera de Talamanca, Costa Rica. *Zootaxa* 2088: 1–14.
- Colli GR, Bastos RP, Araújo AFB. 2002. The character and dynamics of the Cerrado herpetofauna. Pp. 223–241 In: *The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna*. Editors, Oliveira PS, Marquis RJ. Columbia University Press, New York, New York, USA. 409 p.
- Coloma LA, Ron SR, Jungfer K, Grant T, Cisneros-Heredia DF, Solís F, Ibáñez R, Chaves G, Savage JM, Jaramillo C, et al. 2008. *Gastrotheca cornuta*. *The IUCN Red List of Threatened Species* 2008: e.T55329A11294031.
- Crump ML, Scott N. 1994. Visual encounter surveys. Pp. 84–92 In: *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Editors, Heyer WR, Donnelly MR, McDiarmid RW, Hayek LC, Foster MS. Smithsonian Institution Press, Washington, DC, USA. 364 p.
- Dixo M, Martins M. 2008. Are leaf-litter frogs and lizards affected by edge effects due to forest fragmentation in Brazilian Atlantic forest? *Journal of Tropical Ecology* 24(5): 551–554.
- Donnelly M, Guyer C. 1994. Patterns of reproduction and habitat use in an assemblage of Neotropical hylid frogs. *Oecologia* 98: 291–302.
- Duellman WE. 1970. *Hylid Frogs of Middle America*. Monographs of the Museum of Natural History. University of Kansas, Lawrence, Kansas, USA. 448 p.
- Duellman WE. 1978. The biology of an equatorial herpetofauna in Amazonian Ecuador. *Miscellaneous Publications, University of Kansas, Museum of Natural History* 65: 1–352.
- Duellman WE. 1999a. Distribution patterns of amphibians in South America. Pp. 255–328 In: *Patterns of Distribution of Amphibians. A Global Perspective*. Editor, Duellman WE. The Johns Hopkins University Press, Baltimore, Maryland, USA. 648 p.
- Duellman WE. 1999b. *Patterns of Distribution of Amphibians. A Global Perspective*. The Johns Hopkins University Press, Baltimore, Maryland, USA. 648 p.
- Duellman WE. 2001. *Hylid Frogs of Middle America*. New edition. Society for the Study of Amphibians and Reptiles, Ithaca, New York, USA. 1,170 p.

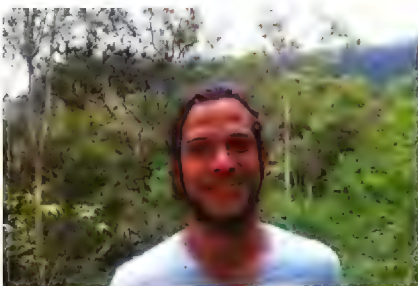
- Duellman WE. 2005. *Cusco Amazónico. The Lives of Amphibians and Reptiles in an Amazonian Rainforest*. Cornell University Press, Ithaca, New York, USA. 433 p.
- Faivovich J, Pereyra MO, Luna MC, Hertz A, Blotto BL, Vásquez-Almazán CR, McCranie JR, Sánchez DA, Baêta D, Araujo-Viera K, et al. 2018. On the monophyly and relationships of several genera of Hylini (Anura: Hylidae: Hylinae), with comments on recent taxonomic changes in hylids. *South American Journal of Herpetology* 13: 1–33.
- Ferreira GC, Sturaro MJ, Peloso PLV. 2017. Amphibians and reptiles from Floresta Nacional de Pau-Rosa, Amazonas, Brazil: an important protected area at the heart of Amazonia. *Acta Amazonica* 47: 259–268.
- Frost DR. 2019. Amphibian species of the World: an online reference. Version 6.0. American Museum of Natural History, New York, New York, USA. Available: <http://research.amnh.org/herpetology/amphibia/index.html> [Accessed: 16 March 2019].
- Funk WC, Caminer M, Ron SR. 2012. High level of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B, Biological Sciences* 279: 1,806–1,814.
- García-Rodríguez A, Chaves G, Benavides-Varela C, Puschendorf R. 2011. Where are the survivors? Tracking relictual populations of endangered frogs in Costa Rica. *Diversity and Distributions* 18: 204–212.
- Gray AR, Bland AW. 2016. Notes on the reproduction of the endemic Costa Rican toad, *Incilius chompipe* (Anura: Bufonidae). *Mesoamerican Herpetology* 3(2): 464–467.
- Guyer C, Donnelly MA. 2005. *Amphibians and Reptiles of La Selva, Costa Rica, and the Caribbean Slope*. University of California Press, Berkeley, California, USA. 420 p.
- Haddad CF, Prado CP. 2005. Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. *The American Institute of Biological Sciences Bulletin* 55(3): 207–217.
- Hayes MP, Pounds J, Timmerman WW. 1989. An annotated list and guide to the amphibians and reptiles of Monteverde, Costa Rica. *Herpetological Circular* 17: 1–67.
- Hertz A, Hauenschild F, Lotzkat S, Köhler G. 2012. A new golden frog species of the genus *Diasporus* (Amphibia, Eleutherodactylidae) from the Cordillera Central, western Panama. *ZooKeys* 196: 23–46.
- Hof C, Araújo MB, Jetz W, Rahbek C. 2011. Additive threats from pathogens, climate, and land-use change for global amphibian diversity. *Nature* 480: 516–519.
- Holdridge LR. 1967. *Life Zone Ecology*. Revised edition. Tropical Science Center, San José, Costa Rica. 149 p.
- IUCN. 2019. The IUCN Red List of Threatened Species. Version 2018-1. Available: <http://www.iucnredlist.org> [Accessed: 23 November 2019].
- IUCN SSC Amphibian Specialist Group. 2019. *Ecnomiohyla veraguensis*. The IUCN Red List of Threatened Species 2019: e.T85341790A90129135.
- Kubicki B. 2007. *Glass Frogs of Costa Rica*. Editorial INBIO, San José, Costa Rica. 304 p.
- Kubicki B. 2008. Amphibian diversity in Guayacán, Limón province, Costa Rica. *Brenesia* 69: 35–42.
- Kubicki B, Salazar S. 2015. Discovery of the golden-eyed fringe-limbed treefrog, *Ecnomiohyla bailarina* (Anura: Hylidae), in the Caribbean foothills of southeastern Costa Rica. *Mesoamerican Herpetology* 2: 76–86.
- La Marca E, Lips KR, Lötters S, Puschendorf R, Ibáñez R, Rueda-Almonacid JV, Schulte R, Marty, C, Castro F, Manzanilla-Puppo J, et al. 2005. Catastrophic population declines and extinctions in neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica* 37: 190–201.
- Laurencio D, Malone JH. 2009. The amphibians and reptiles of Parque Nacional Carara, a transitional herpetofaunal assemblage in Costa Rica. *Herpetological Conservation and Biology* 4: 120–131.
- Leenders T. 2016. *Amphibians of Costa Rica. A Field Guide*. Zona Tropical Press, Ithaca, New York, USA and London, United Kingdom. 531 p.
- Lehtinen RM, Lannoo MJ, Wassersug RJ. 2004. Phytotelm-breeding anurans: past, present, and future research. *Miscellaneous Publications, Museum of Zoology, University of Michigan* 193: 1–9.
- Lima JD. 2008. A herpetofauna do Parque Nacional do Montanhas do Tumucumaque, Amapá, Brasil, Expedições I a V. Pp. 38–50 In: *Inventários Biológicos Rápidos no Parque Nacional Montanhas do Tumucumaque, Amapá, Brasil*. RAP Bulletin of Biological Assessment 48. Editor, Bernard E. Conservation International, Arlington, Virginia, USA. 151 p.
- Lima JRF, Lima JD, Lima SD, Silva RBL, Andrade GV. 2017. Amphibians found in the Amazonian Savanna of the Rio Curiaú Environmental Protection Area in Amapá, Brazil. *Biota Neotropica* 17: e20160252.
- Lips KR, Green DE, Papendick R. 2003. Chytridiomycosis in wild frogs from southern Costa Rica. *Journal of Herpetology* 37: 215–218.
- Lopes-Santos D, Pereira S, Edmar-Pereira V, Vaz-Silva W. 2014. Amphibians and reptiles from southeastern Goiás, central Brazil. *Check List* 10(1): 131–148.
- Lynch JD. 2005. Discovery of the richest frog fauna in the world – an exploration of the forest to the north of Leticia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 29(113): 581–588.
- McDiarmid RW, Savage JM. 2005. The herpetofauna of the Rincón area, Península de Osa, Costa Rica, a Central American lowland evergreen forest site. Pp. 366–427 In: *Ecology and Evolution in the Tropics, a Herpetological Perspective*. Editors, Donnelly

- MA, Crother BI, Guyer C, Wake MH, White ME. University of Chicago Press, Chicago, Illinois, USA. 675 p.
- Morales VR, McDiarmid RW. 1996. Annotated checklist of the amphibians and reptiles of Pakitza, Manu National Park Reserve Zone, with comments on the herpetofauna of Madre de Dios, Peru. Pp. 503–522 In: *The Biodiversity of Southeastern Peru*. Editors, Wilson DE, Sandoval A. Smithsonian Institution Press, Washington, DC, USA. 679 p.
- Myers CW. 1969. Ecological geography of cloud forest in Panama. *American Museum of Natural History Novitates* 2372: 1–28.
- NatureServe, IUCN SSC Amphibian Specialist Group. 2013. *Duellmanohyla uranochroa*. *The IUCN Red List of Threatened Species* 2013: e.T55314A3028798.
- Nogueira C, Colli GR, Martins M. 2009. Local richness and distribution of the lizard fauna in natural habitats of the Brazilian Cerrado. *Austral Ecology* 34: 83–96.
- Peloso PLV. 2010. A safe place for amphibians? A cautionary tale on the taxonomy and conservation of frogs, caecilians, and salamanders in the Brazilian Amazonia. *Zoologia* 27: 667–673.
- Pereira-Júnior AP, Costa-Campos CE, Araújo AS. 2013. Composição e diversidade de anfíbios anuros do campus da Universidade Federal do Amapá. *Biota Amazônia* 3(1): 13–21.
- Pounds JA, Fogden MPL, Campbell JH. 1999. Biological response to climate change on a tropical mountain. *Nature* 398: 611–615.
- Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Fogden MPL, Foster PN, La Marca E, Masters KL, Merino-Viteri A, Puschendorf R, et al. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439: 161–167.
- Pounds J, Bolaños F, Solís F, Ibáñez R, Chaves G, Savage JM, Jaramillo C, Fuenmayor Q. 2008a. *Pristimantis altae*. *The IUCN Red List of Threatened Species* 2008: e.T56406A11470632.
- Pounds J, Bolaños F, Solís F, Ibáñez R, Chaves G, Savage JM, Jaramillo C, Fuenmayor Q, Castro F, Grant T, et al. 2008b. *Pristimantis caryophyllaceus*. *The IUCN Red List of Threatened Species* 2008: e.T56497A11475825.
- Puschendorf R, Hoskin CJ, Cashins SD, McDonald K, Skerratt LF, Vanderwal J, Alford RA. 2011. Environmental refuge from disease-driven amphibian extinction. *Conservation Biology* 25: 956–964.
- Queiroz SS, Silva AR, Reis FM, Lima JD, Lima JRF. 2011. Anfíbios de uma área de castanhal da Reserva Extrativista do Rio Cajari, Amapá. *Biota Amazônia* 1: 1–18.
- Ron SR, Merino-Viteri A, Ortiz A. 2018. *Anfibios del Ecuador*. Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador. Available: <https://bioweb.bio/faunaweb/amphibiaweb> [Accessed: 23 November 2019].
- Rovito SM, Devitt TJ, Devitt SC. 2015. First survey of the amphibians and reptiles of the Nectandra Cloud Forest Reserve, Alajuela, Costa Rica. *Check List* 11: 1,570.
- Ryan MJ, Scott NJ, Cook JA, Willink B, Chaves G, Bolaños F, Garcia-Rodriguez A, Latella IM, Koerner SE. 2015. Too wet for frogs: changes in a tropical leaf litter community coincide with la Niña. *Ecosphere* 6(1): 1–10.
- Salazar S. 2015. Redescubrimiento de la rana marsupial *Gastrotheca cornuta* (Anura: Hemiphractidae) en Costa Rica. *Brenesia* 83–84: 81–82.
- Santos-Barrera G, Pacheco J, Mendoza-Quijano F, Bolaños F, Chaves G, Daily GC, Ehrlich PR, Ceballos G. 2008. Diversity, natural history, and conservation of amphibians and reptiles from the San Vito Region, southwestern Costa Rica. *Revista de Biología Tropical* 6: 755–778.
- Schluter A, Icochea J, Pérez JM. 2004. Amphibians and reptiles of the lower Río Llullapichis, Amazonian Peru: updated species list with ecological and biogeographical notes. *Salamandra* 40(2): 141–160.
- Sasa M, Solórzano A. 1995. The reptiles and amphibians of Santa Rosa National Park, Costa Rica, with comments about the herpetofauna of xerophytic areas. *Herpetological Natural History* 3: 113–126.
- Sasa M, Chaves G, Porras LW. 2010. Costa Rica's herpetofauna: conservation status and future perspectives. Pp. 510–603 In: *Conservation of Mesoamerican Amphibians and Reptiles*. Editors, Wilson LD, Townsend JH, Johnson JD. Eagle Mountain Publishing, Eagle Mountain, Utah, USA. 812 p.
- Savage JM. 2002. *The Amphibians and Reptiles of Costa Rica: a Herpetofauna between Two Continents, between Two Seas*. University of Chicago Press, Chicago, Illinois, USA and London, United Kingdom. 934 p.
- Scott NJ. 1976. The abundance and diversity of the herpetofauna of tropical forest litter. *Biotropica* 8: 41–58.
- Seber GAF. 1986. A review of estimating animal abundance. *Biometrics* 42: 267–292.
- SINAC. 2018. *Plan de manejo, Parque internacional la Amistad - Talamanca*. Sistema Nacional de Áreas de Conservación, San Jose, Costa Rica. Available: <http://www.sinac.go.cr/ES/planmanejo/Plan%20Manejo%20ACLAC/Parque%20Internacional%20La%20Amistad.pdf> [Accessed: 5 January 2019].
- Solís F, Ibáñez R, Savage JM, Jaramillo C, Fuenmayor Q, Kubicki B, Pounds J, Chaves G, Jungfer K, Lips K. 2008. *Agalychnis lemur*. *The IUCN Red List of Threatened Species* 2008: e.T55855A11381418.
- Solórzano A, Lamar WW, Porras L. 1998. The marsupial frog (*Gastrotheca cornuta*; Amphibia, Hylidae) in Isthmian Central America. *Revista Biología Tropical*

- 45(4): 1,675–1,677.
- Streicher JW, Crawford AJ, Edwards CW. 2009. Multilocus molecular phylogenetic analysis of the montane *Craugastor podiciferus* species complex (Anura: Craugastoridae) in Isthmian Central America. *Molecular Phylogenetics and Evolution* 53: 620–630.
- Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1,783–1,786.
- Vaz-Silva W, Oliveira RM, Gonzaga AFN, Pinto KC, Poli CF, Bilce TM, Penhacek M, Wronski L, Martin J, Junqueira TG, et al. 2015. Contributions to the knowledge of amphibians and reptiles from Volta Grande do Xingu, northern Brazil. *Brazilian Journal of Biology* 75: 205–218.
- Verdade VK, Valdujo PH, Carnaval AC, Schiesari L, Toledo LF, Mott T, Andrade GV, Eterovick PC, Menin M, Pimenta BV, et al. 2012. A leap further: the Brazilian Amphibian Conservation Action Plan. *Alytes* 29: 28–43.
- Wake DB, Vredenburg VT. 2008. Colloquium paper: Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 105: 11,466–11,473.
- Wake DB. 1987. Adaptive radiation of salamanders in Middle American cloud forests. *Annals of the Missouri Botanical Garden* 74(2): 242–264.
- Wells KD. 2007. *The Ecology and Behavior of Amphibians*. The University of Chicago Press, Chicago, Illinois, USA. 1,148 p.
- Whitfield SM, Bell KE, Philippi T, Sasa M, Bolaños F, Chaves G, Savage JM, Donnelly MA. 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of the National Academy of Sciences of the United States of America* 104(20): 8,352–8,356.
- Whitfield SM, Alvarado G, Abarca J, Zumbado H, Zúñiga I, Wainwright M, Kerby J. 2017. Differential patterns of *Batrachochytrium dendrobatidis* infection in relict amphibian populations following severe disease-associated declines. *Diseases of Aquatic Organisms* 126: 33–41.
- Young BE, Lips KR, Reaser JK, Ibanez R, Salas AW, Cedeño JR, Coloma LA, Ron S, La Marca E, Meyer JR, et al. 2001. Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* 15: 1,213–1,223.
- Zimmerman BL, Rodrigues MT. 1990. Frogs, snakes, and lizards for the INPA-WWF Reserves near Manaus, Brazil. Pp. 426–454 In: *Four Neotropical Rainforests*. Editor, Gentry AH. Yale University Press, New Haven, Connecticut, USA and London, United Kingdom. 627 p.



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Gerardo Chaves is a biologist from the University of Costa Rica. Gerardo's degree thesis focused on the arrivals of the Olive Ridley Sea Turtles, but most of his professional work has focused on the ecology and taxonomy of the Costa Rican herpetofauna. Since 1992, his research activity has focused on understanding the decline of amphibian populations in Mesoamerica and on filling the herpetofauna inventory gaps in several areas of Costa Rica, mainly across the Talamanca Mountain Range. Since 1997, Gerardo has worked in the Museum of Zoology of the University of Costa Rica in the herpetofauna collection. He has published several journal articles related to the ecology and taxonomy of Neotropical herpetofauna. His conservation efforts are related to the sustainable use of the sea turtle eggs project on "arribadas" and collaboration with IUCN in the evaluation of the Red List for Costa Rica and Mesoamerica, for both reptiles and amphibians. Gerardo is currently chair of the IUCN Amphibian Specialist Group in Costa Rica.

Extreme frog diversity in Costa Rica



Alejandro Acuña is a professional in Ecotourism Management, and he has worked as a coordinator of biodiversity projects in several national parks with the National System of Conservancy Areas (SINAC). Alejandro is a naturalist guide at Veragua Rainforest and research assistant of the Veragua Foundation.



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Three new species of day geckos (Reptilia: Gekkonidae: *Cnemaspis* Strauch, 1887) from isolated granite cave habitats in Sri Lanka

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Abstract.—Three new day gecko species of the genus *Cnemaspis* Strauch, 1887 are described from three isolated granite cave habitats with rock walls in Bambaragala (Ratnapura District), Dimbulagala (Polonnaruwa District), and Mandaramnuwara (Nuwara-Eliya District) in Sri Lanka based on morphometric and meristic characters. All of these new species are assigned to the *kandiana* clade based on morphology. These species are small (28–35 mm SVL) in size and may be differentiated from all other Sri Lankan congeners by a suite of distinct morphometric and meristic characters. Each of these species described herein are categorized as Critically Endangered (CR) under IUCN Red List criteria. At the microhabitat scale, they are restricted to wet, cool, and shady granite caves and rock outcrops in isolated forested areas with limited anthropogenic disturbance. Further, these habitats are located in all three main bioclimatic zones (wet, intermediate, dry) and all three geographic penepains (first, second, third) of Sri Lanka. Due to their restricted distributions (as point endemics), the habitats of these specialist species are vulnerable to fragmentation, edge effects, and anthropogenic activities. Therefore, these isolated forest patches in Sri Lanka are in need of special conservation attention and management.

Keywords. Climate condition, endangered species, habitat specialist, isolated forest, point endemic, range restriction, systematics, taxonomy

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Introduction

Taxonomic descriptions and phylogenetic revisions in the past decade have rapidly increased the number of day gecko species recognized in the genus *Cnemaspis*, bringing the global species richness to more than 155 (Karunarathna et al. 2019a,b; Uetz et al. 2019a). Con-

sequently, *Cnemaspis* ranks as the second most diverse gecko genus in the world, next to *Cyrtodactylus* (Grismer et al. 2014; Uetz et al. 2019a). However, extensive molecular phylogenetic analyses have questioned the monophyly of *Cnemaspis* which is represented by three geographically disjunct groups from South Asia, Tropical Africa, and Southeast Asia (Gamble et al. 2012; Py-

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ron et al. 2013a; Zheng and Wiens 2016). *Cnemaspis* geckos are diminutive, slender-bodied geckos that possess prominent forward and upwardly-directed eyes with round pupils, broad flattened heads, and elongate slender digits that are bent at an angle with entire subdigital lamellae (Vidanapathirana et al. 2014; Wood et al. 2017; Karunarathna et al. 2019a). These geckos are adapted for a scansorial mode of life, with most being rupicolous, while a few are arboreal or ground-dwelling with crepuscular behavior (Das 2005). They appear to be microhabitat specialists with occupancy limited to shaded surfaces of rocks, caves, trees, abandoned buildings, buildings associated with caves, wattle and daub houses, and rock walls within suitable habitats where the cryptic morphology and body coloration help them camouflage with their surroundings (Smith 1935; Karunarathna et al. 2019b).

Much like continental south Asia, as well as the Indo-Malayan realm, the species richness of *Cnemaspis* in Sri Lanka has grown rapidly by at least eight-fold, from four to 33 species (Deraniyagala 1953; Manamendra-Arachchi et al. 2007; Wickramasinghe and Munindradasa 2007; Karunarathna and Ukuwela 2019). As such, *Cnemaspis* has become the most diverse gecko genus on the island, with 100% endemism. Through molecular phylogenetic analyses of mitochondrial and nuclear DNA, Agarwal et al. (2017) demonstrated the presence of two distinct *Cnemaspis* clades in Sri Lanka (*kandiana* and *podihuna*), and indicated the presence of cryptic diversity within four species (*C. alwisi* Wickramasinghe and Munindradasa 2007; *C. kumarsinghei* Wickramasinghe and Munindradasa 2007; *C. latha* Manamendra-Arachchi, Batuwita, and Pethiyagoda 2007, and *C. podihuna* Deraniyagala 1944). The aforementioned studies emphasized the need for detailed studies on *Cnemaspis* taxonomy using a combination of both morphological characteristics and molecular phylogenetics. As indicated by recent studies in Sri Lanka, the faunistic surveys of under-explored habitats followed by rigorous phylogenetic analyses will further augment the species richness of *Cnemaspis* (Bauer et al. 2007; Agarwal et al. 2017; Karunarathna et al. 2019b). In light of this, we conducted field excursions in various isolated localities in Sri Lanka. Here, we describe three new *Cnemaspis* species (based on morphometric and meristic characters) discovered from three such sites which span all three bioclimatic zones and geological peninsulas of Sri Lanka.

Materials and Methods

Field sampling and specimens. Field surveys across 122 different locations in Sri Lanka covered several geographic areas (e.g., dry zone, intermediate zone, and wet zone). At each location, gecko species found were surveyed and documented with special attention on the focal genus. On average, 12 surveyor-hours per location were devoted to the survey. Museum acronyms follow Sabaj (2016) and Uetz et al. (2019b). The type materials discussed in this

paper are deposited in the National Museum of Sri Lanka (NMSL), Colombo, Sri Lanka. Specimens were caught by hand and were photographed in life. They were euthanized using halothane and fixed in 10% formaldehyde for two days, washed in water, and then transferred to 70% ethanol for long-term storage. Tail tips were collected as tissue samples before fixation and were stored in 95% ethanol under relatively cool conditions (20–25 °C). For comparison, 424 *Cnemaspis* specimens (catalogued and uncatalogued) representing all recognized Sri Lankan species were examined, including all type specimens housed at the National Museum, Sri Lanka (NMSL), The Natural History Museum, London (BMNH), and in the private collections of Anslem de Silva (ADS) and Aaron Bauer (AMB), which had been deposited in the NMSL. Specimens that formerly belonged to the Wildlife Heritage Trust (WHT) collection and bear WHT numbers are currently deposited in the NMSL, catalogued under their original numbers. Specimens in this study were collected during a survey of lizards in Sri Lanka under permit numbers WL/3/2/1/14/12 and WL/3/2/42/18 (a and b), issued by the Department of Wildlife Conservation, and under permit numbers FRC/5 and FRC/6, issued by the Forest Department of Sri Lanka. Additional information on the morphology and natural history of Sri Lankan *Cnemaspis* species was extracted from the relevant literature (Bauer et al. 2007; Manamendra-Arachchi et al. 2007; Wickramasinghe and Munindradasa 2007; Vidanapathirana et al. 2014; Wickramasinghe et al. 2016; Batuwita and Udugampala 2017; Agarwal et al. 2017; Batuwita et al. 2019; Karunarathna et al. 2019a,b; de Silva et al. 2019). Assignment of unidentified specimens to the three new species was based on their morphometric and meristic characters (Tables 1–9), color patterns, and geographic isolation (Fig. 1; Table 10). The new species described in the present paper are completely new and have not been included in previous phylogenies of the genus (see Agarwal et al. 2017; Karunarathna et al. 2019b).

Morphometric characters. Forty morphometric measurements were taken using a Mitutoyo digital Vernier calliper (to the nearest 0.1 mm), and detailed observations of scales and other structures were made through Leica Wild M3Z and Leica EZ4 dissecting microscopes. The following symmetrical morphometric characters were taken on the left side of the body: eye diameter (ED), horizontal diameter of eye ball; orbital diameter (OD), greatest diameter of orbit; eye to nostril length (EN), distance between anteriormost point of orbit and posterior border of nostril; snout length (ES), distance between anteriormost point of orbit and tip of snout; snout to nostril length (SN), distance between tip of snout and anteriormost point of nostril; nostril width (NW), maximum horizontal width of nostrils; eye to ear distance (EE), distance between posterior border of eye and anteriormost point of ear opening; snout to axilla distance (SA), distance between axilla and tip of snout;



Fig. 1. Currently known distribution of *Cnemaspis dissanayakai* sp. nov. (square) from Dimbulagala; *Cnemaspis kawminiae* sp. nov. (circle) from Mandaramnuwara; and *Cnemaspis kotagamai* sp. nov. (triangle) from Bambaragala, Sri Lanka.

ear length (EL), maximum length of ear opening; interorbital width (IO), shortest distance between left and right supraciliary scale rows; inter-ear distance (IE), distance across head between the two ear openings; head length (HL), distance between posterior edge of mandible and tip of snout; head width (HW), maximum width of head between the ears and the orbits; head depth (HD), maximum height of head at the level of the eye; jaw length (JL), distance between tip of snout and corner of mouth; internarial distance (IN), smallest distance between inner margins of nostrils; snout to ear distance (SED), distance between tip of snout and anteriormost point of ear; upper-arm length (UAL), distance between axilla and the angle of the elbow; lower-arm length (LAL), distance from elbow to wrist with palm flexed; palm length (PAL), distance between wrist (carpus) and tip of longest finger excluding the claw; length of digits I–V of manus (DLM), distance between juncture of the basal phalanx with the adjacent digit and the tip of the digit, excluding the claw; snout-vent length (SVL) distance between tip of snout and anterior margin of vent; trunk length (TRL), distance between axilla and groin; trunk width (TW),

maximum width of body; trunk depth (TD), maximum depth of body; femur length (FEL), distance between groin and knee; tibia length (TBL), distance from knee to heel with ankle dorsiflexed; heel length (HEL), distance between ankle (tarsus) and tip of longest toe (excluding the claw) with both foot and tibia flexed; length of pedal digits I–V (DLP), distance between juncture of the basal phalanx with the adjacent digit and the digit tip, excluding the claw; tail length (TAL), distance between anterior margin of the vent and tail tip; tail base depth (TBD), maximum height of tail base; tail base width (TBW), widest point of tail base.

Meristic characters. Thirty discrete characters were observed and recorded using Leica Wild M3Z and Leica EZ4 dissecting microscopes on both the left (L) and the right (R) sides of the body (reported in the form L/R): number of supralabials (SUP) and infralabials (INF) between the first labial scale and corner of mouth; number of interorbital scales (INOS), between left and right supraciliary scale rows; number of postmentals (PM) bounded by chin scales, 1st infralabial on the left and right and the mental; number of chin scales (CHS), scales touching medial edge of infralabials and mental between juncture of 1st and 2nd infralabials on the left and right; number of supranasal (SUN) scales between nares; presence of the postnasal (PON) scales posterior to naris; presence of the internasal (INT) scale between supranasals; number of supraciliary scales (SUS) above eye; number of scales between eye and tympanum (BET) from posteriormost point of orbit to anteriormost point of tympanum; number of canthal scales (CAS), number of scales from posteriormost point of naris to anteriormost point of orbit; total lamellae on manus I–V (SLM) counted from first proximal enlarged scansor greater than twice width of the largest palm scale, to distalmost lamella at tip of digits; number of dorsal paravertebral granules (PG) between pelvic and pectoral limb insertion points along a straight line immediately left of vertebral column; number of midbody scales (MBS) from the center of mid-dorsal row diagonally towards the ventral scales; number of midventral scales (MVS) from the first scale posterior to the mental to last scale anterior to vent; number of belly scales (BLS) across venter between the lowest rows of granular dorsal scales; total lamellae on pes I–V (SLP), counted from first proximal enlarged scansor greater than twice the width of the largest heel scale, to distalmost lamella at tip of digits; number of precloacal pores (PCP) anterior to the cloaca; number of femoral pores (FP) present on femur; numbers of non-pored proximal femoral scales (PFS) counted from proximal ends of femoral pore rows to precloacal pores; numbers of non-pored distal femoral scales (DFS) counted from distal ends of femoral pore rows to knee; interfemoral scales (IFS) number of non-pored scales between femoral pores on both femurs. Additional evaluations included the texture [keeled (KD) or smooth (SM)] of the ventral scales, the

texture [heterogeneous (HET) or homogeneous (HOM)] of the dorsal scales, the number of spinous scales on the flanks (FLSP), and characteristics such as appearance of the caudal scales (except in specimens with regenerated tails). Coloration was determined from digital images of living specimens and also from direct observations in the field.

Distribution and natural history. The new species described herein were collected during field surveys conducted in various habitats (e.g., dry mixed semi-evergreen forest and tropical wet-evergreen) of Sri Lanka (Fig. 1; Table 10). During these surveys, behavioral and other aspects of natural history of the focal species were observed through opportunistic and non-systematic means. The ambient and substrate temperatures were measured using a standard thermometer and an N19 Q1370 infrared thermometer (Dick Smith Electronics, Shanghai, China), respectively. The relative humidity and light intensity were measured with a QM 1594 multifunction environment meter (Digitek Instruments Co., Ltd., Hong Kong, China). To record elevation and georeference species locations, an eTrex® 10 GPS (Garmin) was used. Sex was determined by the presence in males (M) or absence in female (F) of hemipenial bulges, and precloacal and femoral pores. The conservation status of each new species was evaluated using the 2001 IUCN Red List Categories and Criteria version 3.1 (IUCN 2012).

Statistical analyses of morphometric characteristics.

Principal Component Analyses (PCA) were performed with the conventional singular value decomposition method using variance-covariance structure as the cross-products matrix to extract 10 principal components (package: PCAMethods, function:pca; Stacklies et al. 2007). The species used for these analyses are: *Cnemaspis dissanayakai* **sp. nov.**, *Cnemaspis ingerorum*, *Cnemaspis kallima*, *Cnemaspis kawminiae* **sp. nov.**, *Cnemaspis kotagamai* **sp. nov.**, *Cnemaspis kumarasinghei*, *Cnemaspis latha*, and *Cnemaspis gotaimbarai* groups due to their close resemblances. All morphometric measurements of the three new species were normalized to the snout-vent length (SVL). The matrix containing

original morphometric variables were Pareto scaled (square-root unit variance) and centered. Subsequently, pairwise ordination plots were generated for the first four principal components (PC), which explained nearly 80% of the cumulative variance, where each individual PC accounted for at least 8% of the overall variance. To visualize species separation in the ordination space, convex hulls were placed around PC scores corresponding to each species (Wickham 2016). In addition, PC loadings were examined against each of the selected PC axes to understand the relationships between the original morphometric variables and the PC axes. This examination also revealed which morphometric variables were most distinct among the different species. In addition to the collective analyses of the eight aforementioned congeners, three separate PCAs were run which focused on three species groups based on their close morphological resemblances: (1) *Cnemaspis kotagamai* **sp. nov.**, *Cnemaspis ingerorum*, and *Cnemaspis kallima*; (2) *Cnemaspis dissanayakai* **sp. nov.**, *Cnemaspis latha*, and *Cnemaspis kumarasinghei*; and (3) *Cnemaspis kawminiae* **sp. nov.**, *Cnemaspis gotaimbarai*, and *Cnemaspis kumarasinghei*. The aforementioned analyses used statistical program R (R Core Team 2019) and RStudio integrated development environment (R Studio Team 2018). Ordination plots were produced using the following statistical applications and R packages: PAST version 3.14 and ggplot2 (Hammer et al. 2001).

Results

Analyses of morphometric data for all eight species.

The PCA resulted in 10 PCs that accounted for 98% of the variability of the original morphometric variables; among these, PC 1–4 (35.64%, 19.48%, 14.35%, and 8.70%, respectively) cumulatively explained 78% of the overall variability (Fig. 2). Trunk and upper-arm lengths had greater loadings on PC1 while tail length, orbital diameter, snout-to-nostril length, and the lengths of the 4th and 5th pedal digits had greater loadings on PC2. Heel length, snout-to-axilla length, eye diameter, inner-ear distance, eye-to-nostril distance, trunk width, and length of the 4th finger had higher loadings on PC3; whereas trunk

Table 1. Principal component scores and corresponding species.

Species	Average scores for principal components					
	PC1	PC2	PC3	PC4	PC5	PC6
<i>C. kotagamai</i>	0.31	0.52	0.31	0.12	0.16	0.00
<i>C. dissanayakai</i>	-0.50	0.20	0.17	-0.22	0.01	-0.10
<i>C. kawminiae</i>	0.40	-0.05	-0.23	-0.27	0.13	0.34
<i>C. kumarasinghei</i>	0.12	-0.28	0.09	-0.02	0.25	-0.21
<i>C. gotaimbarai</i>	0.44	-0.47	0.20	0.06	-0.20	-0.06
<i>C. ingerorum</i>	0.15	0.24	-0.63	0.10	-0.09	-0.19
<i>C. latha</i>	-0.60	-0.03	0.025	0.37	-0.03	0.17
<i>C. kallima</i>	-0.33	-0.13	0.07	-0.14	-0.23	0.03

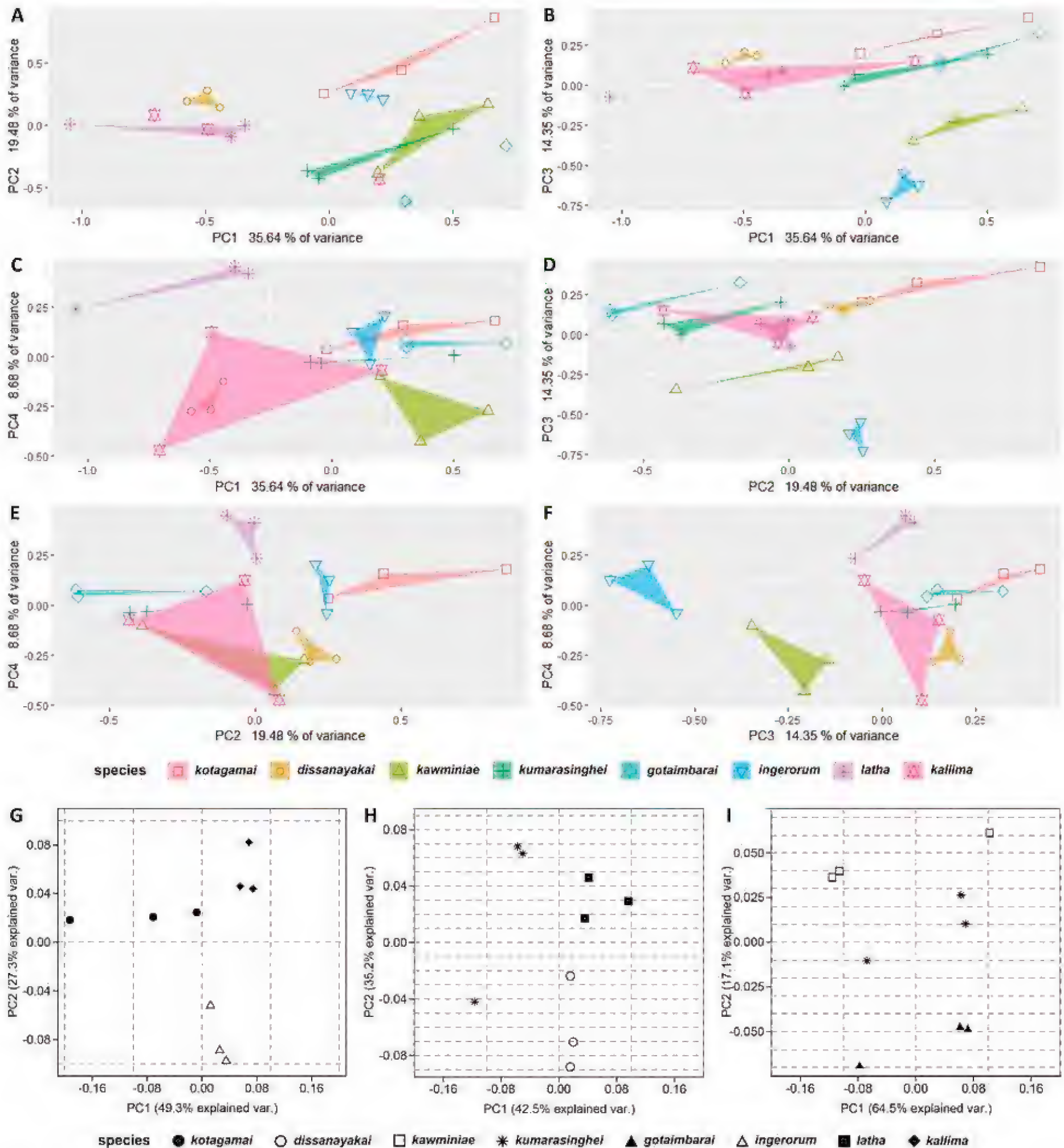


Fig. 2. (A–F) PCA Ordination plots for multivariate morphometric analyses (all pairwise ordination plots for PC1 through PC4 are shown), (G) Scatter plot of PCA between *Cnemaspis kotagamai* sp. nov. (filled circles), *Cnemaspis ingerorum* (triangles), and *Cnemaspis kallima* (filled diamonds), (H) Scatter plot of PCA between *Cnemaspis dissanayakai* sp. nov. (circles), *Cnemaspis latha* (filled squares), and *Cnemaspis kumarasinghei* (stars), (I) Scatter plot of PCA between *Cnemaspis kawminiae* sp. nov. (squares), *Cnemaspis gotaimbarai* (filled triangles), and *Cnemaspis kumarasinghei* (stars).

and tail lengths had higher loadings on PC4 (Tables 1–2). The ordination for PC1 and PC2 provided the strongest evidence for morphometric-based species separation—except for *Cnemaspis kawminiae*, *C. kumarasinghei*, *C. kallima*, and *C. latha*, whose separation was most evident in ordination between PC1 and PC4 (Fig. 2). In addition, PC1 and PC3 supported separation of species based on morphometrics relatively well.

Analyses of morphometric data for separate species groups. PCA of morphometric measurements of *Cnemaspis kotagamai* sp. nov., *Cnemaspis ingerorum*, and *Cnemaspis kallima* indicated the presence of three well separated species (Fig. 2). *Cnemaspis kotagamai* sp. nov. and *Cnemaspis kallima* were clearly separated from the PC2 axis, while *Cnemaspis ingerorum* and *Cnemaspis kallima* were separated along PC1. The PC1 and PC2 axes

Three new species of *Cnemaspis* from Sri Lanka

Table 2. Morphometric variables and corresponding PC loadings.

Measurements	PC1	PC2	PC3	PC4
ED	-0.14	0.02	0.15	0.00
OD	-0.22	0.21	0.06	-0.03
EN	-0.01	-0.14	0.16	-0.04
ES	-0.08	-0.05	-0.12	-0.19
SN	-0.13	0.18	0.03	0.06
NW	0.02	0.04	-0.07	-0.04
EE	-0.10	-0.05	0.03	-0.10
SA	-0.15	-0.40	0.18	-0.15
EL	-0.08	0.14	-0.02	-0.13
IO	-0.22	0.05	0.07	0.06
IE	-0.20	0.02	0.15	-0.03
HL	-0.18	-0.18	0.05	-0.27
HW	-0.16	-0.11	-0.13	0.04
HD	0.08	-0.14	-0.24	-0.07
JL	-0.20	-0.10	-0.17	0.06
IN	-0.16	0.11	0.05	0.03
SED	-0.23	0.08	-0.01	0.12
UAL	-0.29	-0.07	0.03	0.15
LAL	-0.15	-0.26	-0.03	0.18
PAL	-0.04	0.07	-0.28	-0.13
DLM -1	-0.06	-0.02	-0.03	-0.27
DLM-2	-0.01	-0.06	0.00	-0.22
DLM-3	-0.10	-0.03	0.08	-0.17
DLM-4	-0.15	-0.06	0.14	-0.18
DLM-5	-0.17	0.02	0.12	-0.06
TRL	0.22	-0.13	-0.25	-0.48
TW	-0.22	0.11	0.14	-0.15
TD	-0.16	0.11	0.10	-0.09
FEL	-0.16	-0.07	0.05	-0.13
TBL	-0.20	-0.03	-0.11	-0.17
HEL	-0.01	0.06	-0.48	0.22
DLP-1	-0.04	0.07	-0.31	-0.14
DLP-2	-0.24	0.13	-0.20	-0.10
DLP-3	-0.19	-0.03	-0.15	-0.11
DLP-4	-0.19	0.16	-0.24	0.04
DLP-5	-0.22	0.21	-0.20	-0.02
TAL	-0.15	-0.62	-0.20	0.24
TBW	-0.14	-0.03	-0.01	0.22
TBD	-0.11	0.05	0.01	0.21

Table 3. Morphometric data of holotype and two paratypes of *Cnemaspis kotagamai* **sp. nov.** from Bambaragala, Ratnapura District, Sri Lanka.

Measurements	NMSL 2019.15.01	NMSL 2019.15.02	NMSL 2019.15.03
	Holotype (Male)	Paratype (Male)	Paratype (Female)
SVL	29.8	31.1	32.6
TRL	12.6	12.3	12.5
TW	5.4	5.3	5.4
TD	3.4	3.4	3.4
TAL	33.5	33.8	31.1
TBW	3.3	3.1	2.9
TBD	2.9	2.9	2.7
ED	1.9	1.9	1.8
OD	3.2	3.3	3.1
EN	2.8	2.8	2.7
ES	3.6	3.5	3.4
SN	1.6	1.6	1.6
NW	0.2	0.3	0.2
EE	2.5	2.5	2.3
SA	12.9	12.7	11.8
EL	0.8	0.7	0.7
IO	3.6	3.6	3.2
IE	3.8	3.7	3.8
HL	8.3	8.3	8.2
HW	4.5	4.4	4.5
HD	2.8	2.6	2.7
JL	4.9	4.8	4.9
IN	1.6	1.6	1.4
SED	8.7	8.6	8.7
UAL	3.8	3.7	3.7
LAL	3.4	3.3	3.5
PAL	3.2	3.2	3.2
DLM (i)	1.4	1.4	1.3
DLM (ii)	1.9	1.8	1.8
DLM (iii)	2.5	2.5	2.6
DLM (iv)	2.9	2.7	2.8
DLM (v)	2.3	2.3	2.3
FEL	5.8	5.8	5.7
TBL	5.2	5.1	4.9
HEL	4.8	4.5	4.7
DLP (i)	1.2	1.2	1.2
DLP (ii)	2.1	1.9	2.1
DLP (iii)	2.9	2.8	2.7
DLP (iv)	3.8	3.9	3.7
DLP (v)	3.5	3.3	3.5

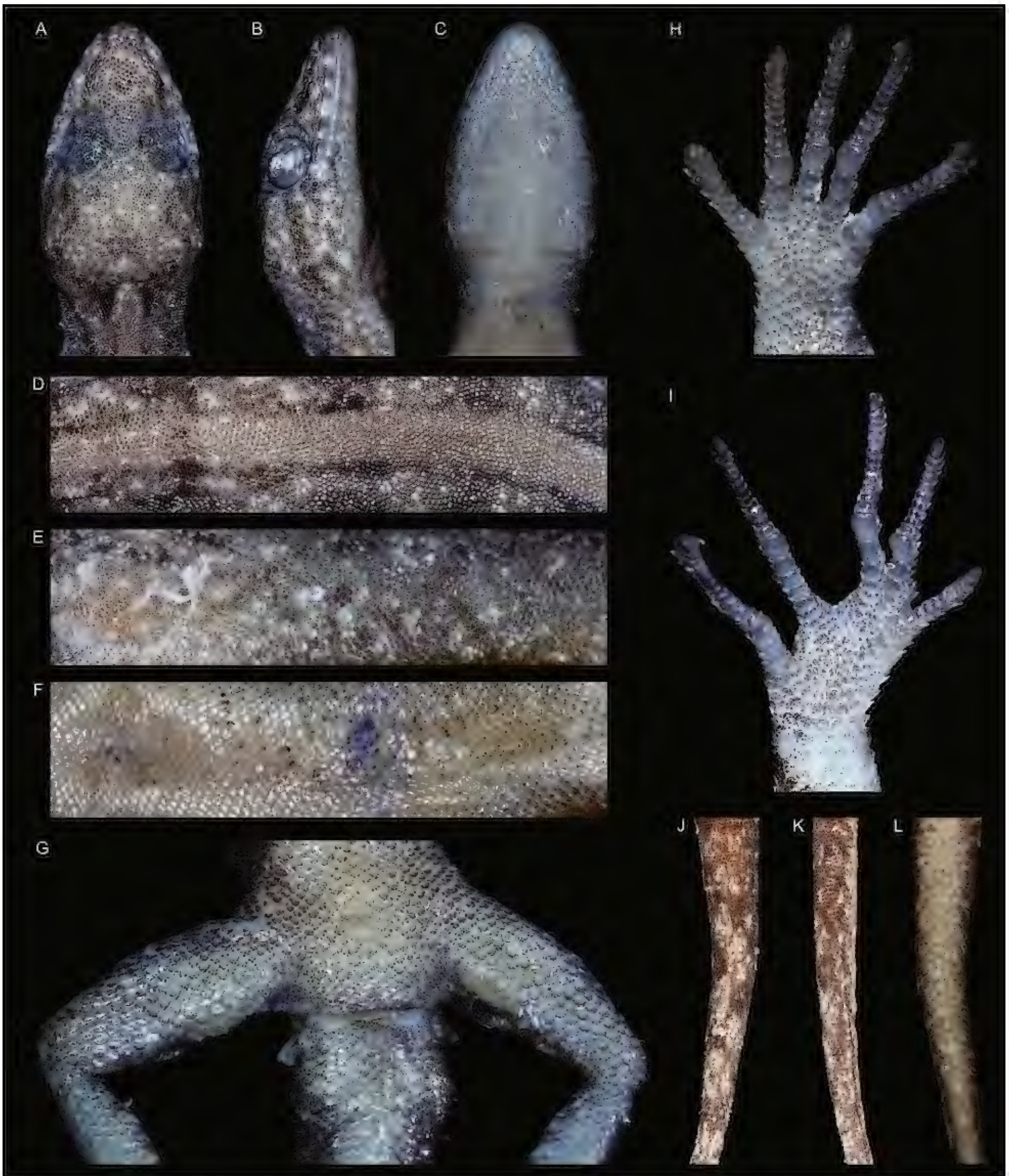


Fig. 3. Holotype male of *Cnemaspis kotagamai* **sp. nov.** (NMSL 2018.15.01). (A) Dorsal head, (B) lateral head, (C) ventral head, (D) heterogeneous dorsal scales, (E) scales on lateral surface of trunk, (F) smooth ventral scales, (G) cloacal characters with pre-cloacal pores and femoral pores, (H) lamellae on manus, (I) lamellae on pes, (J) keeled dorsal scalation of tail, (K) lateral side of tail, and (L) very small smooth subcaudals. Photos: Suranjan Karunaratna.

explained 49.5% and 27.3% of the observed variation, respectively. Analysis of morphometric measurements of *Cnemaspis dissanayakai* **sp. nov.**, *Cnemaspis latha*, and *Cnemaspis kumarasinghei* similarly indicated the presence of three well separated species (Fig. 2). *Cnemaspis dissanayakai* **sp. nov.** and *Cnemaspis kumarasinghei*

were well separated in the PC1 axis while the former was clearly separated from *Cnemaspis latha* in the PC2 axis. The PC1 and PC2 axes explained 42.5% and 32.5% of the observed variation, respectively. PCA analysis of the morphometric measurements of *Cnemaspis kawminiae* **sp. nov.**, *Cnemaspis gotaimbarai*, and *Cnemaspis kuma-*



Fig. 4. Holotype male of *Cnemaspis kotagamai* sp. nov. (NMSL 2018.07.01) in life *in-situ* in Bambaragala. (A) Dorsal view of the full body, and (B) ventral view with scattered yellow coloration. Photos: Suranjan Karunarathna.

rasinghei indicated the presence of three well separated species (Fig. 2). *Cnemaspis kawminiae* sp. nov. was clearly separated from *Cnemaspis gotaimbarai* along the PC2 axis, while it was distinguished from *C. kumarasinghei* also in the PC2 axis. The PC1 and PC2 axes explained 64.5% and 17.1% of the observed variation, respectively.

Systematics

***Cnemaspis kotagamai* sp. nov.** Karunarathna, de Silva, Botejue, Surasinghe, Wickramasinghe, Ukuwela & Bauer

Kotagama's Day Gecko (English)

Kotagamage Diva-seri Hoona (Sinhala)

Kotagamavin Pahalpalli (Tamil)

Figs. 3–5; Tables 3–4

urn:lsid:zoobank.org:act:35801F43-7148-4590-BE38-421AC8905646

Holotype. NMSL 2019.15.01, adult male, 29.8 mm SVL (Fig. 3), collected from a granite cave Bambaragala, Pal-

lebedda, Ratnapura District, Sabaragamu Province, Sri Lanka (6.512978°N, 80.750306°E, WGS1984; elevation 127 m; around 1100 hrs) on 18 January 2019 by Suranjan Karunarathna and Anslem de Silva.

Paratypes. NMSL 2019.15.02, adult male, 31.1 mm SVL, and NMSL 2019.15.03, adult female, 32.6 mm SVL, collected from a granite cave in Bambaragala, Pallebedda, Ratnapura District, Sabaragamuwa Province, Sri Lanka (6.517261°N, 80.752692°E, WGS1984; elevation 132 m; around 1200 hrs) on 18 January 2019 by Suranjan Karunarathna and Anslem de Silva.

Diagnosis. *Cnemaspis kotagamai* sp. nov. may be readily distinguished from its Sri Lankan congeners by a combination of the following morphological and meristic characteristics as well as color patterns: maximum SVL 32.6 mm; dorsum with heterogeneous, smooth intermixed with weakly keeled granular scales; 2/2 supranasals, one internasal, 2/2 postnasals; 3–4 enlarged postmentals; postmentals bounded by 5–6 chin scales; chin, gular, pectoral, and abdominal scales smooth, subimbricate; 21–22 belly scales across midbody; 6–7 well-devel-

Table 4. Meristic data of holotype and two paratypes of *Cnemaspis kotagamai* **sp. nov.** from Bambaragala, Ratnapura District, Sri Lanka.

Counts	NMSL 2019.15.01	NMSL 2019.15.02	NMSL 2019.15.03
	Holotype (Male)	Paratype (Male)	Paratype (Female)
FLSP (L/R)	6/7	6/6	7/7
SUP (L/R)	8/8	8/8	7/8
INF (L/R)	7/7	8/7	7/7
INOS	31	29	31
PM	4	4	3
CHS	6	5	6
SUN (L/R)	2/2	2/2	2/2
PON (L/R)	2/2	2/2	2/2
INT	1	1	1
SUS (L/R)	12/12	14/13	12/12
BET (L/R)	22/22	21/19	21/22
CAS (L/R)	11/10	11/10	10/10
TLM (i) (L/R)	9/9	10/9	10/10
TLM (ii) (L/R)	12/12	12/11	12/12
TLM (iii) (L/R)	14/14	14/13	13/13
TLM (iv) (L/R)	15/15	14/14	14/13
TLM (v) (L/R)	12/12	11/12	12/12
PG	114	119	116
MBS	84	79	81
MVS	134	137	131
BLS	21	21	22
TLP (i) (L/R)	9/9	8/9	8/8
TLP (ii) (L/R)	14/14	13/14	14/14
TLP (iii) (L/R)	16/16	16/16	15/16
TLP (iv) (L/R)	17/17	17/18	18/17
TLP (v) (L/R)	16/16	15/15	16/15
PCP	1	1	-
FP (L/R)	5/5	4/4	-
PFS (L/R)	12/13	11/12	-
DFS (L/R)	2/2	4/6	-

oped tubercles on posterior flank; 114–119 paravertebral granules linearly arranged; one precloacal pore, 4–5 femoral pores in males, separated by 11–13 unpored proximal femoral scales, 2–6 unpored distal femoral scales; 131–137 ventral scales; 79–84 midbody scales; subcaudals smooth, median row comprising an irregular series of diamond-shaped, small scales; 7–8 supralabials; 7–8 infralabials; 13–15 total lamellae on 4th digit of manus, and 17–18 total lamellae on 4th digit of pes.

Comparisons with other Sri Lankan species. Among species of the *C. kandiana* clade *sensu* Agarwal et al. (2017), *Cnemaspis kotagamai* **sp. nov.** differs by having heterogeneous (*versus* homogeneous) dorsal scales

from *C. amith* Manamendra-Arachchi et al. 2007, *C. gotaimbarai* Karunaratna et al. 2019b, *C. kumarasinghei* Wickramasinghe and Munindradasa 2007, *C. latha* Manamendra-Arachchi et al. 2007, and *C. nandimithrai* Karunaratna et al. 2019b; it can also be diagnosed from *C. butewai* Karunaratna et al. 2019b, *C. kandiana* (Kelaart, 1852), *C. kivulegedarai* Karunaratna et al. 2019b, *C. menikay* Manamendra-Arachchi et al. 2007, *C. pava* Manamendra-Arachchi et al. 2007, *C. pulchra* Manamendra-Arachchi et al. 2007, *C. retigalensis* Wickramasinghe and Munindradasa 2007, *C. samanensis* Wickramasinghe and Munindradasa 2007, *C. silvula* Manamendra-Arachchi et al. 2007, *C. tropidogaster* (Boulenger, 1885), and *C. upendrai* Manamendra-Arachchi et al. 2007 by having smooth (*versus* keeled) pectoral scales. The new species differs from *C. kallima* Manamendra-Arachchi et al. 2007 by having more midbody scales (79–84 *versus* 67–74), presence of more paravertebral granules (114–119 *versus* 99–107), by having fewer precloacal pores (1 *versus* 3–4), and having fewer tubercles on the posterior flank (6–7 *versus* 12–15). It differs from *C. ingororum* Batuwita et al. 2019 by having more ventral scales (131–137 *versus* 88–95) and more paravertebral granules (114–119 *versus* 93–101).

Among species of the *C. podihuna* clade *sensu* Agarwal et al. (2017), *Cnemaspis kotagamai* **sp. nov.** differs by the absence of clearly enlarged, hexagonal or subhexagonal subcaudal scales from the following species: *C. alwisi* Wickramasinghe and Munindradasa 2007, *C. anslemi* Karunaratna and Ukuwela 2019, *C. gemunu* Bauer et al. 2007, *C. godagedarai* de Silva et al. 2019, *C. hitihami* Karunaratna et al. 2019b, *C. kandambyi* Batuwita and Udugampala 2017, *C. kohukumburai* Karunaratna et al. 2019b, *C. molligodai* Wickramasinghe and Munindradasa 2007, *C. nilgala* Karunaratna et al. 2019, *C. phillipsi* Manamendra-Arachchi et al. 2007, *C. podihuna* Deraniyagala, 1944, *C. punctata* Manamendra-Arachchi et al. 2007, *C. rajakarunai* Wickramasinghe et al. 2016, *C. rammalensis* Vidanapathirana et al. 2014, and *C. scalpensis* (Ferguson 1877).

Description of Holotype (NMSL 2019.15.01). An adult male, 29.8 mm SVL and 33.5 mm TAL. Body slender, relatively long (TRL/SVL ratio 42.2%). Head relatively small (HL/SVL ratio 28.0% and HL/TRL ratio 66.2%), narrow (HW/SVL ratio 15.3% and HW/HL ratio 54.6%), depressed (HD/SVL ratio 9.5% and HD/HL ratio 33.9%), and distinct from neck. Snout relatively long (ES/HW ratio 78.4% and ES/HL ratio 42.8%), less than twice eye diameter (ED/ES ratio 52.5%), more than half length of jaw (ES/JL ratio 72.1%), snout slightly concave in lateral view; eye relatively small (ED/HL ratio 22.5%), larger than the ear (EL/ED ratio 44.4%), pupil rounded; orbit length greater than eye to ear distance (OD/EE ratio 127.8%) and length of IV digit of manus (OD/DLM IV ratio 111.8%); supraocular ridges moderately developed; ear opening small (EL/HL ratio 10.0%), deep, taller than



Fig. 5. General habitat of *Cnemaspis kotagamai* sp. nov. at Bambaragala isolated forest hill, Ratnapura District, Sri Lanka. (A) Rock outcrop habitat, (B) abandoned cave building, and (C) deep and tall granite tunnel. Photos: Madhava Botejue.

wide, larger than nostrils; two rows of scales separate orbit from supralabials; interorbital distance is greater than snout length (IO/ES ratio 101.7%), shorter than head length (IO/HL ratio 43.5%); eye to nostril distance greater than the eye to ear distance (EN/EE ratio 109.5%).

Dorsal surface of the trunk with smooth scales intermixed with weakly keeled heterogeneous granules, 114 paravertebral granules; 134 midventral scales, smooth; 84 midbody scales; 6/7 weakly developed tubercles on the flanks; ventrolateral scales irregular, enlarged; granules on snout smooth and raised, larger than those on interorbital and occipital regions; canthus rostralis nearly absent, 11/10 smooth oval scales from eye to nostril; scales of the interorbital region circular and smooth; tubercles present both on the sides of the neck and around the ear; ear opening vertically oval, slanting from anterodorsal to posteroventral, 22/22 scales between anterior margin of the ear opening and the posterior margin of the eye. Supralabials 8/8, infralabials 7/7, becoming smaller towards the gape. Rostral scale wider than long, partially divided (80%) by a median groove, contact with first supralabial. Nostrils separated by 2/2 enlarged supranasals with one internasal; no enlarged scales behind the supranasals. Nostrils oval, dorsolaterally orientated, not in contact with first supralabials; 2/2 postnasals, smooth, larger than nostrils, partially in contact with first supralabial.

Mental sub-rhomboid in shape, as wide as long, posteriorly in contact with four enlarged postmentals (smaller than mental, and larger than chin scales); postmentals in contact and bordered posteriorly by six unkeeled chin scales (smaller than nostrils), in contact with the 1st infralabial; ventral scales smaller than chin scales. Smooth, rounded, juxtaposed scales on the chin and the gular region; pectoral and abdominal scales smooth, subimbricate to imbricate towards precloacal region, abdominal scales slightly larger than dorsals; 21 belly scales across venter; smooth scales around vent and base of tail, subimbricate; one precloacal pore; 5/5 femoral pores; 12/13 unpored proximal femoral scales on each side; 2/2 enlarged distal femoral scales. Regenerated tail of holotype a little longer than the snout-vent length (TAL/SVL ratio 112.7%); hemipenial bulge greatly swollen (TBW 3.3 mm), heterogeneous scales on the dorsal aspect of the tail directed backwards, spine-like tubercles present at the base of tail; tail with 4–5 enlarged flattened obtuse scales forming whorls; a large, blunt post-cloacal spur on each side, dorsoventrally flattened and narrow; subcaudals smooth and small, subrhomboidal, arranged in a single median series.

Forelimbs very short, slender (LAL/SVL ratio 11.4% and UAL/SVL ratio 12.7%); hind limbs long, tibia shorter than femur (TBL/SVL ratio 17.3% and FEL/SVL ratio

19.4%). Anterior surface of upper arm with keeled and less imbricate scales; dorsal, posterior, and ventral surface smooth, scales of the anterior surface twice as large as those of the other surfaces; anterior and dorsal surfaces of lower arm with keeled and less imbricate scales, ventral and posterior surfaces with unkeeled imbricate scales, scales on the anterior surface of upper arm and lower arm twice the size of those of other aspects. Scales on dorsal and ventral surfaces of femur smooth, those on anterior and posterior surfaces keeled, scales on the ventral surface twice the size of those of other aspects. Dorsal, anterior, and posterior surfaces of tibia with keeled and weakly imbricate scales, ventral surface with smooth, subimbricate scales, scales of the ventral surface twice as large as those on other aspects. Dorsal and ventral surfaces of manus and pes with keeled granules; dorsal surfaces of digits with granular scales. Digits elongate and slender with inflected distal phalanges, all bearing slightly recurved claws. Subdigital lamellae entire (except divided at first interphalangeal joint), unnotched; total lamellae on manus (left/right): digit I (9/9), digit II (12/12), digit III (14/14), digit IV (15/15), digit V (12/12); total lamellae on pes (left/right): digit I (9/9), digit II (14/14), digit III (16/16), digit IV (17/17), digit V (16/16); interdigital webbing absent; relative length of left manual digits: I (1.4 mm), II (1.9 mm), V (2.3 mm), III (2.5 mm), IV (2.9 mm); relative length of left pedal digits: I (1.2 mm), II (2.1 mm), III (2.9 mm), V (3.5 mm), IV (3.8 mm).

Variation of the type series. The SVL of adult specimens in the type series of *Cnemaspis kotagamai* **sp. nov.** ($n = 3$) ranges from 29.8 to 32.6 mm; interorbital scales 29–31; supraciliaries above the eye 12–14; scales from eye to tympanum 19–22; canthal scales 10–11; tubercles on posterior flank 6–7; chin scales 5–6; ventral scales 131–137 (Tables 3–4); midbody scales 79–84; paravertebral granules 114–119; belly scales across venter 21–22; femoral pores in males 4–5; unpored proximal femoral scales in males 11–13; unpored distal femoral scales in males 2–6; total lamellae on digit of the manus: digit I (9–10), digit II (11–12), digit III (13–14), digit IV (13–15), digit V (11–12); total lamellae on digit of the pes: digit I (8–9), digit II (13–14), digit III (15–16), digit IV (17–18), digit V (15–16).

Color of living specimens. Dorsum of head, body, and limbs generally brown; one broad, yellow vertebral stripe running from occiput to tail (Fig. 4); five irregular blackish-brown paravertebral blotches present; occipital area with a ‘W’-shaped dark marking. Tail dark brown dorsally, with 11 faded black cross-bands; pupil circular and black with the surrounding margins yellow and orange, supraciliaries yellowish; two black postorbital stripes on each side; an oblique black line between the eye and nostril on either side; supralabials and infralabials yellowish with tiny black spots; chin and gular scales dirty white, without dark spots; pectoral, abdominal, cloacal, and subcaudal scales immaculate cream; dorsum of

limbs with faded black patches; manus and pes alternating black and cream-white crossbands.

Color of preserved specimens. Dorsally blackish-brown with five distinct dark, irregular brown blotches (Fig. 3); supralabials and infralabials dirty white; chin and gular scales grey; ventral surface uniformly dirty white in color, with some scales on thigh, tail base, and arms with dark brown margins.

Etymology. The specific epithet is an eponym Latinized (*kotagamai*) in the masculine genitive singular, honoring prominent Sri Lankan scientist (ornithologist), Sarath Wimalabandara Kotagama (Emeritus Professor of the University of Colombo) for his valuable contributions towards biodiversity conservation and management in Sri Lanka.

Distribution and natural history. The type locality, Bambaragala forest (6.509086–6.522369°N and 80.742731–80.759386°E; Ratnapura District, Sabaragamuwa Province), is located in the lowland (southern intermediate bioclimatic zone) where tropical moist semi-evergreen forests comprise the dominant vegetation type (Gunatilleke and Gunatilleke 1990). The forest acreage is ~50 ha and relatively isolated by anthropogenically-altered flat lands. Bambaragala lies at an elevation of 110–178 m asl. The mean annual rainfall of 1,500–2,000 mm is received mainly during the southwest monsoon (May–September), while the mean annual temperature is 27.8–29.6 °C. Bambaragala is rich in granite rock caves with over 30 identified caves. *Cnemaspis kotagamai* **sp. nov.** appeared to be a very rare species in Bambaragala, as only five individuals were recorded during the survey. This species was located in a granite cave on vertical surfaces, 4 m in height, within the forested area (Fig. 5). The microhabitat of *C. kotagamai* **sp. nov.** was poorly illuminated (light intensity: 385–469 Lux), relatively moist (relative humidity: 71–88%), canopy-shaded (canopy cover: 65–80%), and relatively cool (ambient temperature: 29.8–31.3 °C and substrate temperature: 27.8–28.6 °C). The new species was sympatric with several other gecko species: *Gehyra mutilata*, *Hemidactylus depressus*, *H. frenatus*, and *H. parvimaclatus*. No eggs were observed.

Conservation status. Application of the IUCN Red List criteria indicates that *C. kotagamai* **sp. nov.** is Critically Endangered (CR), due to having an area of occupancy (AOO) <10 km² (four locations, 0.13 km² in total assuming a 100 m radius around the georeferenced location) and an extent of occurrence (EOO) <100 km² (0.37 km²) in Sabaragamuwa Province [Applicable criteria B2-b (iii)].

Remarks. *Cnemaspis kotagamai* **sp. nov.** most closely resembles *C. ingerorum* (southern dry zone, ~85 m asl) and *C. kallima* (northern wet zone, ~600 m asl) morphologically, the type localities of these species are separated by ~63 km (Sandagala in Tissamaharamaya) and ~115 km (Gammaduwa in Matale) straight line distances from



Fig. 6. Holotype male of *Cnemaspis dissanayakai* sp. nov. (NMSL 2018.20.01). (A) Dorsal head, (B) lateral head, (C) ventral head, (D) homogeneous dorsal scales, (E) scales on lateral surface of trunk, (F) smooth ventral scales, (G) cloacal characters with precloacal pores and femoral pores, (H) lamellae on manus, (I) lamellae on pes, (J) smooth dorsal scalation of tail, (K) lateral side of tail, and (L) very small smooth subcaudals. Photos: Suranjan Karunarathna.

Bambaragala in Palledbedda (Fig. 1). Also see the comparison with other species for more details.

***Cnemaspis dissanayakai* sp. nov.** Karunarathna, de Silva, Madawala, Karunarathna, Wickramasinghe, Ukuwela & Bauer
Dissanayaka's Day Gecko (English)

Dissanayakage Diva-seri Hoona (Sinhala)
Dissanayakavin Pahalpalli (Tamil)
Figs. 6–8; Tables 5–6

urn:lsid:zoobank.org:act:7ABF9B28-7D04-4296-BDB5-ECEA07B1F965

Holotype. NMSL 2019.20.01, adult male, 28.6 mm SVL

Table 5. Morphometric data of holotype and two paratypes of *Cnemaspis dissanayakai* **sp. nov.** from Dimbulagala, Polonnaruwa District, Sri Lanka.

Measurements	NMSL 2019.20.01	NMSL 2019.20.02	NMSL 2019.20.03
	Holotype (Male)	Paratype (Male)	Paratype (Female)
SVL	28.6	28.2	29.4
TRL	11.1	11.2	11.0
TW	5.5	5.4	5.7
TD	3.6	3.4	3.5
TAL	31.1	31.2	34.4
TBW	2.5	2.7	2.8
TBD	2.2	2.3	2.2
ED	1.9	1.9	1.9
OD	3.3	3.1	3.1
EN	2.4	2.4	2.3
ES	3.6	3.7	3.6
SN	1.3	1.4	1.4
NW	0.2	0.2	0.2
EE	2.5	2.7	2.5
SA	13.1	12.9	12.8
EL	0.8	0.8	0.9
IO	3.6	3.6	3.7
IE	3.8	3.7	3.9
HL	9.0	8.9	8.9
HW	4.3	4.5	4.4
HD	2.4	2.5	2.5
JL	5.5	5.6	5.6
IN	1.6	1.6	1.6
SED	8.1	8.2	8.2
UAL	4.6	4.6	4.6
LAL	4.2	4.2	4.2
PAL	3.2	3.2	3.3
DLM (i)	1.6	1.6	1.7
DLM (ii)	1.8	1.8	1.9
DLM (iii)	2.8	2.7	2.7
DLM (iv)	3.3	3.2	3.4
DLM (v)	2.5	2.4	2.6
FEL	5.6	5.6	5.6
TBL	5.7	5.7	5.5
HEL	3.9	3.8	3.8
DLP (i)	1.5	1.5	1.5
DLP (ii)	3.2	3.2	3.3
DLP (iii)	3.6	3.6	3.7
DLP (iv)	4.1	4.1	4.2
DLP (v)	3.9	3.8	3.7

Table 6. Meristic data of holotype and two paratypes of *Cnemaspis dissanayakai* **sp. nov.** from Dimbulagala, Polonnaruwa District, Sri Lanka.

Counts	NMSL 2019.20.01	NMSL 2019.20.02	NMSL 2019.20.03
	Holotype (Male)	Paratype (Male)	Paratype (Female)
FLSP (L/R)	7/6	7/7	6/6
SUP (L/R)	7/7	7/7	7/7
INF (L/R)	7/7	7/7	7/7
INOS	31	29	29
PM	3	3	3
CHS	7	6	6
SUN (L/R)	2/2	2/2	2/2
PON (L/R)	1/1	1/1	1/1
INT	1	1	1
SUS (L/R)	16/16	16/17	16/15
BET (L/R)	22/23	21/21	21/22
CAS (L/R)	11/12	11/11	11/11
TLM (i) (L/R)	10/10	10/10	10/10
TLM (ii) (L/R)	13/12	12/12	12/12
TLM (iii) (L/R)	13/13	12/13	12/12
TLM (iv) (L/R)	22/21	21/21	21/21
TLM (v) (L/R)	14/14	13/14	14/13
PG	105	107	105
MBS	98	94	95
MVS	118	120	119
BLS	17	17	19
TLP (i) (L/R)	8/8	8/9	8/8
TLP (ii) (L/R)	13/14	13/13	13/13
TLP (iii) (L/R)	16/16	16/15	16/16
TLP (iv) (L/R)	22/21	21-21	21/21
TLP (v) (L/R)	17/16	17/17	17/17
PCP	2	2	-
FP (L/R)	5/4	4/4	-
PFS (L/R)	10/10	11/10	-
DFS (L/R)	7/5	7/7	-

(Fig. 6), collected from a large granite cave in the shaded forest of Dimbulagala, Polonnaruwa District, North-Central Province, Sri Lanka (7.872931°N, 81.135569°E, WGS1984; elevation 129 m; around 1600 hrs) on 12 July 2018 by Suranjan Karunaratna and Anslem de Silva.

Paratypes. NMSL 2019.20.02, adult female, 29.4 mm SVL, and NMSL 2019.20.03, adult male, 28.2 mm SVL, collected from moss covered granite cave in Dimbulagala, Polonnaruwa District, North-Central Province, Sri Lanka (7.851358°N, 81.141675°E, WGS1984; elevation 135 m; around 1200 hrs) on 12 July 2018 by Suranjan Karunaratna and Anslem de Silva.

Diagnosis. *Cnemaspis dissanayakai* **sp. nov.**, may be readily distinguished from its Sri Lankan congeners by a combination of the following morphological and meristic characteristics: maximum SVL 29.4 mm; dorsum with homogeneous, subconical granular scales; one internasal, 2/2 supranasals, 1/1 postnasals; 29–31 interorbital scales; 15–17 supraciliaries, 11–12 canthal scales, 21–23 eye to tympanum scales; three enlarged postmentals; postmentals bounded by 6–7 chin scales; chin with smooth granules, gular, pectoral, and abdominal scales smooth, subimbricate; 17 belly scales across the venter; 6–7 well developed tubercles on posterior flank; 105–107 linearly arranged paravertebral granules; two precloacal pores, 4–5 femoral pores on each side in males separated by 10–11 unpored proximal femoral scales, 5–7 unpored distal femoral scales; 118–120 ventral scales; 94–98 midbody scales; subcaudals smooth, median row small, in an irregular series of diamond-shaped scales; 7/7 supralabials; 7/7 infralabials; 21–22 total lamellae on 4th digit of manus, and 21–22 total lamellae on 4th digit of pes.

Comparisons with other Sri Lankan species. Among species of the *C. kandiana* clade *sensu* Agarwal et al. (2017), *Cnemaspis dissanayakai* **sp. nov.** differs from *C. butewai*, *C. ingerorum*, *C. kallima*, *C. kandiana*, *C. kivulegedarai*, *C. kotagamai* **sp. nov.**, *C. menikay*, *C. pava*, *C. pulchra*, *C. retigalensis*, *C. samanalensis*, *C. silvula*, *C. tropidogaster*, and *C. upendrai* by having homogeneous (*versus* heterogeneous) dorsal scales; from *C. amith* by having smooth (*versus* keeled) pectoral scales; from *C. kumarasinghei*, *C. latha*, and *C. nandimithrai* by having more paravertebral granules (105–107 *versus* 61–68, 72–79, and 95–99, respectively), and from by having more total lamellae on digit IV of manus and digit IV of pes (21–22 *versus* 16–18, 17–18, and 19–20, respectively); from *C. gotaimbarai* by having fewer paravertebral granules (86–92 *versus* 117–121), fewer ventral scales (107–114 *versus* 129–138), and fewer total lamellae on digit IV of manus and digit IV of pes (15–16 *versus* 19–20).

Among species of the *C. podihuna* clade *sensu* Agarwal et al. (2017), *Cnemaspis dissanayakai* **sp. nov.** differs by the absence of clearly enlarged, hexagonal or subhexagonal subcaudal scales from the following species: *C. alwisi*, *C. anslemi*, *C. gemunu*, *C. hitihami*, *C. kandambyi*, *C. kohukumburai*, *C. molligodai*, *C. nilgala*, *C. phillipsi*, *C. podihuna*, *C. punctata*, *C. rajakarunai*, *C. rammalensis*, and *C. scalpensis*.

Description of Holotype (NMSL 2019.20.01). An adult male, 28.6 mm SVL, and 31.1 mm TAL. Body slender, relatively short (TRL/SVL ratio 38.8%). Head relatively long (HL/SVL ratio 31.5% and HL/TRL ratio 81.1%), very narrow (HW/SVL ratio 15.1% and HW/HL ratio 48.0%), depressed (HD/SVL ratio 8.2% and HD/HL ratio 26.2%), and distinct from neck. Snout relatively long (ES/HW ratio 82.2% and ES/HL ratio 39.5%), less than

twice eye diameter (ED/ES ratio 52.8%), more than half length of jaw (ES/JL ratio 65.2%), snout slightly concave in lateral view; eye relatively small (ED/HL ratio 20.8%), twice as large as the ear (EL/ED ratio 43.6%), pupil rounded; orbit length greater than eye to ear distance (OD/EE ratio 131.0%) and also shorter than length of IV digit of manus (OD/DLM IV ratio 99.7%); supraocular ridges not prominent; ear opening very small (EL/HL ratio 9.1%), deep, taller than wide, larger than nostrils; two rows of scales separate orbit from supralabials; interorbital distance slightly shorter than snout length (IO/ES ratio 99.7%), less than half of head length (IO/HL ratio 39.4%); eye to nostril distance slightly shorter than the eye to ear distance (EN/EE ratio 95.2%).

Dorsal surface of trunk with homogeneous, subconical granules; 105 paravertebral granules; 118 mid-ventral scales, smooth; 98 midbody scales; 7/6 well developed tubercles on flanks; ventrolateral scales not enlarged; granules on snout strongly keeled, larger than those on interorbital and occipital regions; canthus rostralis nearly absent, 11/12 smooth round scales from eye to nostril; scales of the interorbital region oval and smooth; 2/2 small and blunt tubercles present on sides of neck, and around ear; ear opening vertically oval, backward slanted, 22/23 scales between anterior margin of ear opening and posterior margin of eye. Supralabials 7/7, infralabials 7/7, becoming smaller towards the gape. Rostral scale wider than long, partially divided (70%) by a median groove, in contact with first supralabial. Nostrils separated by 2/2 enlarged supranasals with one internasal; no enlarged scales behind supranasals. Nostrils oval, dorso-laterally orientated, not in contact with first supralabials; 1/1 postnasals, smooth, larger than nostrils, partially in contact with first supralabial.

Mental subtriangular, as wide as long, posteriorly in contact with three enlarged postmentals (smaller than mental, and larger than chin scales); postmentals in contact and bordered posteriorly by seven smooth chin scales (smaller than nostrils), in contact only with 1st infralabials; ventral scales smaller than chin scales; smooth, rounded, juxtaposed scales on the chin and gular region; pectoral and abdominal scales smooth, subimbricate to imbricate towards precloacal region, abdominal scales slightly larger than dorsals; 17 belly scales across venter; scales around vent and base of tail smooth, subimbricate; two precloacal pores; 5/4 femoral pores; 10/10 unpored proximal femoral scales on each side; 7/5 enlarged distal femoral scales. Original tail of holotype longer than snout-vent length (TAL/SVL ratio 108.7%); tail base greatly swollen (TBW 2.5 mm), heterogeneous scales on dorsum of the tail directed backwards, spine-like tubercles along tail; tail with 4–6 enlarged flattened obtuse scales forming whorls; a small, blunt post-cloacal spur on each side, dorsoventrally flattened and narrow; median series of smooth, irregular, oval to rhomboid subcaudals.

Forelimbs moderately short, slender (LAL/SVL ratio 14.7% and UAL/SVL ratio 15.9%); hind limbs long,

tibia barely longer than the femur (TBL/SVL ratio 19.7% and FEL/SVL ratio 19.6%). Dorsal, anterior, and posterior surfaces of upper arm and lower arm with keeled and less imbricate scales than ventrals, ventral surfaces smooth, less imbricate scales than ventrals, scales of the anterior surface twice as large as those of the other surfaces. Scales on dorsal, posterior, and ventral surfaces of femur smooth and granular, anterior surface with keeled subimbricate scales, anterior surface twice as large as those of the other aspects; dorsal, anterior and posterior surfaces of tibia with keeled and subimbricate scales, ventral scales smooth, subimbricate, twice as large as those of the other limb surfaces. Manus and the pes with keeled granules dorsally and ventrally; dorsum of digits with granular scales; digits elongate and slender with inflected distal phalanges, all bearing slightly recurved claws. Subdigital lamellae entire (except divided at first interphalangeal joint), unnotched; total lamellae on manus (left/right): digit I (10/10), digit II (13/12), digit III (13/13), digit IV (22/21), digit V (14/14); total lamellae on pes (left/right): digit I (8/8), digit II (13/14), digit III (16/16), digit IV (22/21), digit V (17/16); interdigital webbing absent; relative length of digits of left manus: I (1.6 mm), II (1.8 mm), V (2.5 mm), III (2.8 mm), IV (3.3

mm); relative length of digits of left pes: I (1.5 mm), II (3.2 mm), III (3.6 mm), V (3.9 mm), IV (4.1 mm).

Variation of the type series. The SVL of adult specimens in the type series of *Cnemaspis dissanayakai* **sp. nov.** ($n = 3$) ranges from 28.2 to 29.4 mm; interorbital scales 29–31; supraciliaries above the eye 15–17; scales from eye to tympanum 21–23; canthal scales 11–12; tubercles on posterior flank 6–7; chin scales 6–7; ventral scales 118–120; midbody scales 94–98; paravertebral granules 105–107 (Tables 5–6); belly scales across venter 17–19; femoral pores 4–5; unpored proximal femorals 10–11; unpored distal femoral scales 5–7; total lamellae on digit of the manus: digit I (8–9), digit II (13–14), digit III (15–16), digit IV (21–22); total lamellae on digit of the pes: digit I (8–9), digit II (13–14), digit III (15–16), digit IV (21–22), digit V (16–17).

Color of living specimens. Dorsum of the head, body, and limbs generally dull brown, varying from light maroon to light brown, five faded and irregular ‘W’-shaped brown markings on the trunk; 4–5 cream vertebral blotches (Fig. 7); an oblique black line between eye and nostrils on either side, two straight, dark brown postorbital stripes



Fig. 7. Holotype male of *Cnemaspis dissanayakai* **sp. nov.** (NMSL 2018.20.01) in life *in-situ* in Dimbulagala. (A) Dorsal view of the full body, and (B) ventral view with dirty white coloration. Photos: Suranjan Karunaratna.



Fig. 8. General habitat of *Cnemaspis dissanayakai* sp. nov. at Dimbulagala isolated hill forest, Polonnaruwa District, Sri Lanka. (A) Complete view of the mountain, (B) abandoned ancient cave building in Kosgahaulpatha, and (C) deep and tall granite tunnel. Photos: Madhava Botejue and Ashan Geeganage.

extend from eyes posteroventrally, and a faded spot present in the occipital area. Tail grey-pink dorsally, with 5–7 irregular faded brown cross-bands; pupil is circular and black with the surrounding orange, with supraciliaries being light brownish; supralabials dirty whitish dusted with black; infralabials greyish dusted with black; mid-gular scales are yellowish; pectoral, abdominal, cloacal, and subcaudal scales white without markings; dorsum of limbs with irregular brown patches and lines; manus and pes with black and cream cross white stripes on dorsum.

Color of preserved specimens. Dorsum dark brown with grey, faded indistinct irregular brown markings; vertebral blotches cream. Venter dirty white with some scales on throat, abdomen, thigh, tail base, and arms with dark brown margins (Fig. 6).

Etymology. The specific epithet is an eponym Latinized (*dissanayakai*) in the masculine genitive singular, honoring Dissanayaka Mudiyansele Karunaratna (born in Nilgala, Bibila) – father of the first author (Suranjan Karunaratna) for his encouragement, financial support for research, and for allowing SK to pursue his interest in wildlife.

Distribution and natural history. The type locality, Dimbulagala (7.843919–7.876344°N, 81.105603–

81.156442°E), situated in the Polonnaruwa District, North Central Province (northeast dry bioclimatic zone) of Sri Lanka, supports tropical dry-mixed evergreen forests (Gunatilleke and Gunatilleke 1990), and is ~1,000 ha in size. The mean annual rainfall of 1,500–2,000 mm is received mainly during the northeast monsoon (November–February). The mean annual temperature of the area is 28.9–30.2 °C, and its elevation range is 120–250 m asl. According to preliminary investigations, *Cnemaspis dissanayakai* sp. nov. appeared to be very rare in Dimbulagala. The survey of 35 ha recorded two (± 0.1) geckos per surveyor-hour of effort. This species was restricted to rocky surfaces and granite caves in shaded forested areas, and old abandoned buildings inside the forest (Fig. 8). These microhabitats were well-shaded (light intensity: 594–648 Lux), relatively humid (relative humidity: 65–90%), and moderately warm (ambient temperature: 30.2–31.9 °C and substrate temperature 27.5–28.6 °C). The new species was observed to occur in sympatry with the following gecko species: *Calodactylodes illingworthorum*, *Gehyra mutilata*, *Hemidactylus depressus*, *H. frenatus*, *H. huna*, *H. parvumaculatus*, and *H. triedrus*. Older and newly laid eggs were observed in granite rock crevices, usually laid in clusters of three. The eggs were pure white in color and almost spherical in shape (mean diameter 4.9 ± 0.02 mm), with a slightly flattened side attached to the rocky substrate.



Fig. 9. Holotype male of *Cnemaspis kawminiae* **sp. nov.** (NMSL 2018.18.01). (A) Dorsal head, (B) lateral head, (C) ventral head, (D) homogeneous dorsal scales, (E) scales on lateral surface of trunk, (F) smooth ventral scales, (G) cloacal characters with precloacal pores and femoral pores, (H) lamellae on manus, (I) lamellae on pes, (J) smooth dorsal scalation of tail, (K) lateral side of tail, and (L) very small subcaudals. *Photos: Suranjan Karunaratna.*

Conservation status. Application of the IUCN Red List criteria indicates that *C. dissanayakai* **sp. nov.** is Critically Endangered (CR) due to having an area of occupancy (AOO) <10 km² (four locations, 0.13 km² in total assuming a 100 m radius around the georeferenced location) and an extent of occurrence (EOO) <100 km² (4.08 km²) in North Central Province [Applicable criteria B2-b (iii)].

Remarks. *Cnemaspis dissanayakai* **sp. nov.** most closely resembles *C. kumarasinghei* (east intermediate zone) and *C. latha* (southern intermediate zone) morphologically. The type localities of these species are separated by ~105 km (Maragala in Monaragala, ~500 m asl) and ~90 km (Bandarawela in Badulla, ~700 m asl) straight line distances from Dimbulagala in Polonnaruwa (Fig. 1). Also see the comparison with other species for more details.

Three new species of *Cnemaspis* from Sri Lanka

Table 7. Morphometric data of holotype and two paratypes of *Cnemaspis kawminiae* **sp. nov.** from Mandaramnuwara, Nuwara-Eliya District, Sri Lanka.

Measurements	NMSL 2019.18.01	NMSL 2019.18.02	NMSL 2019.18.03
	Holotype (Male)	Paratype (Male)	Paratype (Female)
SVL	33.7	33.2	35.6
TRL	16.4	14.9	16.2
TW	5.5	5.4	5.4
TD	3.4	3.2	3.3
TAL	36.1	42.7	38.0
TBW	3.4	3.4	3.2
TBD	2.9	2.8	2.7
ED	1.5	1.5	1.4
OD	3.1	2.9	2.9
EN	3.1	3.2	3.1
ES	4.3	4.5	4.8
SN	1.2	1.3	1.7
NW	0.3	0.3	0.3
EE	3.2	3.2	3.2
SA	14.9	14.8	14.9
EL	0.9	0.9	0.9
IO	2.9	2.9	2.9
IE	3.7	3.7	3.5
HL	9.9	9.4	10.4
HW	5.5	5.3	5.2
HD	3.8	3.6	3.6
JL	5.9	5.8	5.8
IN	1.3	1.3	1.3
SED	8.3	8.2	8.0
UAL	4.5	4.3	4.3
LAL	4.5	4.5	4.5
PAL	4.6	4.2	4.2
DLM (i)	2.1	2.2	2.2
DLM (ii)	2.5	2.5	2.6
DLM (iii)	2.6	2.7	2.6
DLM (iv)	3.0	2.9	3.0
DLM (v)	2.2	2.2	2.2
FEL	6.5	6.3	6.5
TBL	6.1	6.1	6.1
HEL	5.3	5.8	6.1
DLP (i)	2.1	2.1	2.1
DLP (ii)	3.0	2.9	3.0
DLP (iii)	3.5	3.5	3.5
DLP (iv)	3.8	3.8	3.8
DLP (v)	3.6	3.8	3.6

Table 8. Meristic data of holotype and two paratypes of *Cnemaspis kawminiae* **sp. nov.** from Mandaramnuwara, Nuwara-Eliya District, Sri Lanka.

Counts	NMSL 2019.18.01	NMSL 2019.18.02	NMSL 2019.18.03
	Holotype (Male)	Paratype (Male)	Paratype (Female)
FLSP (L/R)	8/7	7/7	8/7
SUP (L/R)	8/8	8/8	8/7
INF (L/R)	7/7	8/7	7/7
INOS	21	20	22
PM	3	3	3
CHS	5	5	5
SUN (L/R)	2/2	2/2	2/2
PON (L/R)	2/2	2/2	2/2
INT	1	1	1
SUS (L/R)	10/10	10/10	10/9
BET (L/R)	22/20	21/21	22/20
CAS (L/R)	10/11	10/10	10/10
TLM (i) (L/R)	10/9	10/9	10/10
TLM (ii) (L/R)	13/13	12/12	13/12
TLM (iii) (L/R)	14/13	14/14	14/14
TLM (iv) (L/R)	15/14	15/15	14/14
TLM (v) (L/R)	13/14	14/14	14/13
PG	89	92	86
MBS	78	76	76
MVS	107	108	114
BLS	21	17	19
TLP (i) (L/R)	9/9	10/9	10/10
TLP (ii) (L/R)	12/13	12/12	12/12
TLP (iii) (L/R)	16/15	16/16	15/15
TLP (iv) (L/R)	16/16	15/16	15/16
TLP (v) (L/R)	14/14	14/15	15/15
PCP	2	2	-
FP (L/R)	4/4	4/4	-
PFS (L/R)	12/11	12/13	-
DFS (L/R)	7/6	7/7	-

***Cnemaspis kawminiae* sp. nov.** Karunarathna, de Silva, Gabadage, Karunarathna, Wickramasinghe, Ukuwela & Bauer

Kawmini's Day Gecko (English)

Kawminige Divaseri Hoona (Sinhala)

Kawminivin Pahalpalli (Tamil)

Figs. 9–11; Tables 7–8

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Holotype. NMSL 2019.18.01, adult male, 33.7 mm SVL (Fig. 9), collected from a moss-covered granite wall in Mandaramnuwara, bordering Pidurutalagala Mountain range, Nuwara-Eliya District, Central Province, Sri

Lanka (7.033558°N, 80.798794°E, WGS1984; elevation 1,600 m asl, around 1100 hrs) on 14 December 2018 by Suranjan Karunarathna and Anslem de Silva.

Paratypes. NMSL 2019.18.02, adult male, 33.2 mm SVL and NMSL 2019.18.03, adult female, 35.2 mm SVL, collected from a small granite cave Mandaramnuwara, bordering Pidurutalagala Mountain, Nuwara-Eliya District, Central Province, Sri Lanka (7.020600°N, 80.788639°E, WGS1984; elevation 1,658 m asl, around 1400 hrs) collected on 14 December 2018 by Suranjan Karunarathna and Anslem de Silva.

Diagnosis. *Cnemaspis kawminiae* **sp. nov.**, may be readily distinguished from its Sri Lankan congeners by a combination of the following morphological and meristic characteristics: maximum SVL 35.2 mm; dorsum with homogeneous flat granular scales; one internasal, 2/2 supranasals and 2/2 postnasals; 20–22 interorbital scales; 9–10 supraciliaries, 10–11 canthal scales, 20–22 eye to tympanum scales; three enlarged postmentals; postmentals bounded by five chin scales; chin with smooth and round granules, gular, pectoral, and abdominal scales smooth, subimbricate; 17–21 belly scales across the venter; 7–8 weakly developed tubercles on posterior flank; 86–92 linearly arranged paravertebral granules; two precloacal pores in males, 4/4 femoral pores on each side in males separated by 11–13 unpored proximal femoral scales, 6–7 unpored distal femoral scales; 107–114 ventral scales; 76–78 midbody scales; subcaudals smooth, median row small, in an irregular series of sub-rhomboid shaped scales; 7–8 supralabials; 7–8 infralabials; 14–15 total lamellae on 4th digit of manus, and 15–16 total lamellae on 4th digit of pes.

Comparisons with other Sri Lankan species. Among species of the *C. kandiana* clade *sensu* Agarwal et al. (2017), *Cnemaspis kawminiae* **sp. nov.** differs from *C. butewai*, *C. ingerorum*, *C. kallima*, *C. kandiana*, *C. kivulegedarai*, *C. kotagamai* **sp. nov.**, *C. menikay*, *C. pava*, *C. pulchra*, *C. retigalensis*, *C. samanalensis*, *C. silvula*, *C. tropidogaster*, and *C. upendrai* by having homogeneous (*versus* heterogeneous) dorsal scales; from *C. amith* by having smooth (*versus* keeled) pectoral scales; from *C. gotaimbarai*, *C. kumarasinghei*, and *C. dissanayakai* **sp. nov.** by having fewer ventral scales (107–114 *versus* 129–138, 120–134 and 118–120, respectively), and also from *C. kumarasinghei* and *C. dissanayakai* **sp. nov.** by having fewer midbody scales (76–78 *versus* 87–94 and 94–98, respectively), from *C. gotaimbarai* by having fewer paravertebral granules (86–92 *versus* 117–121); from *C. latha* by having more paravertebral granules (86–92 *versus* 72–79), and more belly scales (17–21 *versus* 13–15); from *C. nandimithrai* by having fewer belly scales (17–21 *versus* 25–27) and by having fewer total lamellae on digit IV of pes (15–16 *versus* 19–20).

The new species, *Cnemaspis kawminiae* **sp. nov.**, also clearly differs from the following species of the *C. podihuna* clade *sensu* Agarwal et al. (2017): *C. alwisi*, *C. anslemi*, *C. gemunu*, *C. godagedarai*, *C. hitihami*, *C. kandambyi*, *C. kohukumburai*, *C. molligodai*, *C. nilgala*, *C. phillipsi*, *C. podihuna*, *C. punctata*, *C. rajakarunai*, *C. rammalensis*, and *C. scalpensis* by the absence (*versus* presence) of clearly enlarged, hexagonal or subhexagonal subcaudal scales.

Description of Holotype (NMSL 2019.18.01). An adult male, 33.7 mm SVL, and 36.1 mm TAL. Body slender, relatively long (TRL/SVL ratio 48.7%). Head relatively small (HL/SVL ratio 29.4% and HL/TRL ratio 60.4%), relatively broad (HW/SVL ratio 16.4% and HW/HL ratio 55.6%), weakly depressed (HD/SVL ratio 11.2% and HD/HL ratio 38.1%), and distinct from neck. Snout relatively short (ES/HW ratio 78.4% and ES/HL ratio 43.6%), slightly less than three times eye diameter (ED/ES ratio 34.4%), more than half length of jaw (ES/JL ratio 73.3%), snout slightly concave in lateral view; eye very small (ED/HL ratio 15.0%), larger than ear (EL/ED ratio 59.7%), pupil rounded; orbit length slightly smaller than eye to ear distance (OD/EE ratio 97.2%) and longer than length of IV digit of manus (OD/DLM IV ratio 105.4%); supraocular ridges weakly prominent; ear opening very small (EL/HL ratio 9.0%), deep, taller than wide, larger than nostrils; two rows of scales separate orbit from supralabials; interorbital distance less than snout length (IO/ES ratio 67.7%), head length three times longer than interorbital distance (IO/HL ratio 29.5%); eye to nostril distance subequal to eye to ear distance (EN/EE ratio 98.1%).

Dorsal surface of trunk with homogeneous, flat granular and smooth scales; 112 paravertebral granules; 149 midventral scales, keeled; 69 midbody scales; 6/6 well developed tubercles on flanks; ventrolateral scales irregularly enlarged; granules on snout strongly keeled, larger than those on interorbital and occipital regions; canthus rostralis nearly absent, 9/10 smooth round scales from eye to nostril; scales of the interorbital region oval and smooth; small and blunt tubercles present on sides of neck, and around ear; ear opening vertically oval, slanting from anterodorsal to posteroventral, 20/19 scales between anterior margin of ear opening and the posterior margin of eye. Supralabials 8/7, infralabials 7/7, becoming smaller towards the gape. Rostral scale wider than long, partially divided (90%) by a median groove, in contact with first supralabial. Nostrils separated by 2/2 enlarged supranasals with one internasal, 2/2 postnasals; no enlarged scales behind supranasals. Nostrils oval, dorso-laterally orientated, not in contact with first supralabials.

Mental subtriangular, as wide as long, posteriorly in contact with three enlarged postmentals (smaller than mental, and larger than chin scales); postmentals in contact and bordered posteriorly by five smooth chin scales

(smaller than nostrils), in contact only with 1st and 2nd infralabials; ventral scales smaller than chin scales. Smooth, oval, juxtaposed scales on the chin and gular region; pectoral and abdominal scales smooth, subimbricate to imbricate towards precloacal region, abdominal scales slightly larger than dorsals; 21 belly scales across venter; scales around vent and base of tail smooth, subimbricate; two precloacal pores; 4/4 femoral pores; 12/11 unpored proximal femoral scales on each side; 7/6 enlarged distal femoral scales. Original tail of holotype longer than snout-vent length (TAL/SVL ratio 106.9%); hemipenial bulge greatly swollen (TBW 3.4 mm), homogeneous scales on dorsum of the tail directed backwards, spine-like tubercles along tail; tail with 4–5 enlarged flattened obtuse scales forming whorls; a large, blunt postcloacal spur on each side, dorsoventrally flattened and narrow; a single median series of smooth, irregular, oval to rhomboid subcaudals.

Forelimbs very short, slender (LAL/SVL ratio 13.3% and UAL/SVL ratio 13.3%), upper arm and lower arm equal in size; hind limbs long, tibia slightly shorter than femur (TBL/SVL ratio 18.0% and FEL/SVL ratio 19.3%). Dorsal, anterior, and posterior surfaces of upper arm and lower arm with keeled and less imbricate scales than ventral scales, ventral surfaces with smooth scales, scales of the anterior surface twice as large as those of the other aspects. Scales on anterior and posterior surfaces of femur keeled, dorsal and ventral scales smooth, ventral scales twice as large as those of the other limb surfaces. Scales on dorsal, anterior, and posterior surfaces of tibia keeled, ventral scales smooth, anterior scales twice as large as those of the other limb surfaces. Manus and pes with smooth granules dorsally and ventrally; dorsum of digits with conical granular smooth scales. Digits elongate and slender with inflected distal phalanges, all bearing slightly recurved claws. Subdigital lamellae entire (except divided at first interphalangeal joint), unnotched; total lamellae on manus (left/right): digit I (10/9), digit II (13/13), digit III (14/13), digit IV (15/14), digit V (13/14); total lamellae on pes (left/right): digit I (9/9), digit II (12/13), digit III (16/15), digit IV (16/16), digit V (14/14); interdigital webbing absent; relative length of left manual digits: I (2.1 mm), V (2.2 mm), II (2.5 mm), III (2.6 mm), IV (3.0 mm); relative length of left pedal digits: I (2.1 mm), II (3.0 mm), III (3.5 mm), V (3.6 mm), IV (3.8 mm).

Variation of the type series. The SVL of adult specimens in the type series of *Cnemaspis kawminiae* **sp. nov.** ($n = 3$) ranges from 33.2 to 35.6 mm; interorbital scales 20–22; scales from eye to tympanum 20–22; canthal scales 10–11; supraciliaries 9–10; tubercles on posterior flank 7–8; ventral scales 107–114 (Tables 7–8); midbody scales 76–78, paravertebral granules 86–92, belly scales across venter 17–21; unpored proximal femorals 11–13 in males, unpored distal femoral scales 6–7 in males; total lamellae on digit of the manus: digit I (9–10), digit

II (12–13), digit III (13–14), digit IV (14–15), digit V (13–14); total lamellae on digit of the pes: digit I (9–10), digit II (12–13), digit III (15–16), digit IV (15–16), digit V (14–15).

Color of living specimens. Dorsal body, limb, and tail generally light grey to brown, with an oblique black line in the interorbital area, also between eye and nostril; a wide ‘W’-shaped, black patch on the occipital area with two median cream-white spots; four scattered, double ‘W’-shaped brownish markings on the dorsum of the trunk with tiny irregular stripes, and ten grey brownish blotches along the tail (Fig. 10). Lateral side of limbs and body grey-brown with scattered black spots, and cream colored lateral conical tubercles on tail and trunk. Three straight, dark brown postorbital stripes-downwards and upwards; supraciliaries and nasals greyish brown. Pupil circular and black with the surrounding scales yellowish brown, supralabials and infralabials with a median cream spot. Ventral surfaces of head, body, and limbs beige to cream, but gular area covered in tiny black spots; ventral surface of tail cream colored.

Color of preserved specimens. Dorsum cinnamon brown, with faded double ‘W’-shaped patches on dorsum; irregular tiny brown dots on head; faded brown line between eye and nostrils on both sides, and three brown postorbital stripes on either side (Fig. 9); venter dirty white with some scales on throat, abdomen, thigh, tail base, and arms with dark brown dots.

Etymology. The specific epithet is an eponym Latinized (*kawminiae*) in the feminine genitive singular, honoring Hadunneththi Kawmini Mendis – mother of the first author (Suranjan Karunarathna) for her unconditional love, generous support, and financial support for research.

Distribution and natural history. The type locality, Mandaramnuwara (7.020103–7.039953°N and 80.768794–80.807014°E) in the east wet bioclimatic zone is located at the northern part of Pidurutalagala mountain range (Fig. 11). This area supports tropical montane forest vegetation (Gunatilleke and Gunatilleke 1990) with wet evergreen forest. The core study area was ~300 ha in size, at an elevation range ~1,400–1,800 m asl and annual temperature of 27.4–28.9 °C. *Cnemaspis kawminiae* **sp. nov.** was not abundant in the study area as only five (± 0.1) geckos per surveyor-hour were found in Mandaramnuwara. This species was found on moss covered boulders and rock surfaces in forested areas and well-shaded home gardens with ample woody tree cover (light intensity: 486–592 Lux); as well as rock walls and rock crevices along roads. These habitats were very wet and cool (ambient temperature: 24.2–26.5 °C, substrate temperature: 26.7–28.3 °C, canopy cover: 70–85% and relative humidity: 74–92%). The mean annual rainfall of 3,000–4,000 mm is received mainly during the southwest



Fig. 10. Holotype male of *Cnemaspis kawminiae* sp. nov. (NMSL 2018.18.01) in life *in-situ*. (A) Dorsal view of the full body, and (B) dorsolateral view with labial coloration. Photos: Madhava Botejue.

monsoon (May–September). A total of 26 females, 11 males, and eight juveniles of this species were observed from twelve sites in the Mandaramnuwara area. During July to September, hatchlings, juveniles, and gravid females carrying one or two eggs were observed. Eggs were pure white (mean diameter 5.2 ± 0.02 mm), and almost completely round in shape with a slightly flattened side which was often the side attached to the substrate or between the eggs.

Conservation status. Application of the IUCN Red List criteria indicates that *C. kawminiae* sp. nov. is Critically Endangered (CR) due to having an area of occupancy (AOO) <10 km² (four locations, 0.13 km² in total assuming a 100 m radius around the georeferenced location) and an extent of occurrence (EOO) <100 km² (2.32 km²) in Central Province [Applicable criteria B2-b (iii)].

Remarks. *Cnemaspis kawminiae* sp. nov. most closely resembles *C. kumarasinghei* (east intermediate zone) and *C. gotaimbarai* (northeast dry zone) morphologically. The type localities of these species are separated by ~80 km (Maragala in Monaragala, ~500 m asl) and ~44 km (Kokagala in Padiyathalawa, ~300 m asl) straight line distances from Mandaramnuwara (~1,500 m asl) in Nuwara-Eliya District (Fig. 1). Also see the comparison with other species for more details.

Discussion

The recent renaissance in the taxonomy and systematics of genus *Cnemaspis* has led to a notable increase in species richness, particularly from south and south-eastern Asia, including the Indo-Malayan mainland as well as Indian-oceanic and south-pacific islands (Iskandar et al. 2017; Riyanto et al. 2017; Wood et al. 2017; Karunara-



Fig. 11. General habitats of *Cnemaspis kawminiae* sp. nov. at Mandaramnuwara, Nuwara-Eliya District, Sri Lanka. (A) Complete view of the granite hill at roadside, (B) small granite cave close to the stream, and (C) granite rock wall along the road. Photos: Madhava Botejue.

thna et al. 2019b; Uetz et al. 2019a). With over 160 species, *Cnemaspis* is considered the second-most speciose gecko genus in the world, after *Cyrtodactylus* (Grismer et al. 2014; Present paper). With the inclusion of the three new species described here, species richness of *Cnemaspis*, the most species-rich reptile genus of Sri Lanka, rises to 36 (13 species described in year 2019). Similar phylogenetic radiations with high degrees of endemism via complex evolutionary processes have been documented for snakes, other Gekkonid squamates, and amphibians of Sri Lanka (Bauer et al. 2010; Pyron et al. 2013b; Meegaskumbura et al. 2019). Our study further bolsters the notion that Sri Lanka is a hotspot for reptile diversity and endemism (Bossuyt et al. 2004). Since all three currently described members of this genus are endemic to the island, *Cnemaspis* exhibits the greatest degree of genus-level endemism in Sri Lanka. Sri Lankan *Cnemaspis* species represent two distinct evolutionary lineages, the *kandiana* and *podihuna* clades (Agarwal et al. 2017; Karunarathna et al. 2019b).

The three new species described in this paper have not been included in any previous phylogenies of the genus

(Bauer et al. 2007; Agarwal et al. 2017; Karunarathna et al. 2019b). All of these new species (*C. dissanayakai* sp. nov., *C. kawminiae* sp. nov., and *C. kotagamai* sp. nov.) were assigned to the *C. kandiana* clade based on the presence of small and irregularly shaped subcaudal scales (see Karunarathna and Ukuwela 2019). However, more in-depth phylogenetic studies are necessary to confirm the placement of these three new species within this clade and subgroups (Table 9). Hence, we strongly recommend broader and more robust molecular phylogenetic studies on *Cnemaspis* species, as well as on other gecko species, to identify the true richness within the island. Almost all Sri Lankan *Cnemaspis* species are found within relatively cool, moist habitats (ambient temperature: 24.2–32.3 °C; substrate temperature: 25.2–28.7 °C; relative humidity: 68–92%), with relatively high levels of canopy cover and high-profile mature trees, and shady (canopy cover: 60–90%; light intensity: 385–821 Lux) environments with tall large trees (Karunarathna et al. 2019b). Moreover, all of these new species were found in granite caves or in association with rocky substrates. Such aspects of natural history and microhabitat selec-

Table 9. Key characters of 36 currently known *Cnemaspis* species in Sri Lanka. Abbreviations: mm – Millimeters, SVL – Maximum Snout to vent length, SUB – Subcaudals, SUP – Supralabials, INF – Infralabials, PG – Paravertebral granules, IFS – Interfemoral scales, FLSP – Flank spines, PCP – Precloacal pores, FP – Femoral pores, HET – Heterogeneous, HOM – Homogeneous, KD – Keeled, SM – Smooth.

Species	SVL (mm)	Dorsal	Gular	Pectoral	Abdomen	SUB	SUP	INF	Ventrals	Belly	Midbody	PG	IFS	FLSP	PCP	FP	Lamellae 4 th finger	Lamellae 4 th toe
Clade (1) <i>Cnemaspis kandiana</i> (subcaudals small)																		
Group (1) – <i>tropidogaster</i>																		
<i>C. pava</i>	32.4	HET	KD	KD	KD	Small	7–8	6–7	139–145	22–25	64–75	83–98	–	9–11	2–4	4–5	16–17	18–19
<i>C. pulchra</i>	34.2	HET	KD	KD	KD	Small	7–8	7–8	120–135	24–27	67–73	94–103	–	5–7	3–4	4–6	15–17	17–20
<i>C. samanalen-sis</i>	37.5	HET	KD	KD	KD	Small	8–10	8–9	128–144	19–20	61–67	64–72	–	5–6	3–4	3–5	16–17	18–20
<i>C. silvula</i>	28.6	HET	KD	KD	KD	Small	7–8	7–8	132–139	19–21	73–81	102–113	–	10–15	3–4	4–5	15–16	18–19
<i>C. tropido-gaster</i>	31.7	HET	KD	KD	KD	Small	7–8	7–8	132–146	21–25	92–98	99–106	–	5–7	3–4	4–5	16–17	18–19
<i>C. upendrai</i>	35.2	HET	KD	KD	KD	Small	7–8	7–8	112–128	16–25	69–74	97–102	–	13–15	2–3	4–5	17–18	17–21
Group (2) – <i>kandiana</i>																		
<i>C. butewai</i>	31.8	HET	KD	SM	SM	Small	8	7–8	125–128	23–25	92–98	134–138	–	5–6	3–5	5	16	17–18
<i>C. ingerorum</i>	26.9	HET	SM	SM	SM	Small	7	7	88–95	17–21	62–69	93–101	–	7–9	2	5	13–16	17–18
<i>C. kallima</i>	35.1	HET	SM	SM	SM	Small	7–8	7–8	131–138	19–23	67–74	99–107	–	12–15	3–4	4–5	16–18	18–20
<i>C. kivulege-darai</i>	31.2	HET	SM	SM	SM	Small	7	6–7	109–114	19	69–76	131–133	–	4–5	2	4–5	14	15
<i>C. kotagamai sp. nov.</i>	29.8	HET	SM	SM	SM	Small	7–8	7–8	131–137	21–22	79–84	114–119	–	6–7	1	4–5	13–15	17–18
<i>C. kandiana</i>	34.6	HET	KD	SM	SM	Small	8–9	7–8	119–138	19–20	68–75	86–99	–	5–7	2–4	3–4	12–14	18–20
<i>C. menikay</i>	28.0	HET	KD	SM	SM	Small	7–9	7–8	124–138	20–26	71–79	83–98	–	13–15	1–2	3–4	14–15	15–17
<i>C. retigalensis</i>	30.8	HET	KD	SM	SM	Small	7–8	7–8	121–128	16–20	69–77	82–86	–	4–5	1	3–4	14–15	16–20
Group (3) – <i>amith</i>																		
<i>C. amith</i>	33.0	HOM	KD	SM	SM	Small	7–8	7	123–131	19–21	67–74	79–84	–	4–5	3	3	16–17	18–19
Group (4) – <i>kumarasinghei</i>																		
<i>C. dissanay-akai sp. nov.</i>	29.4	HOM	SM	SM	SM	Small	7	7	118–120	17–19	94–98	105–107	–	6–7	2	4–5	21–22	21–22
<i>C. gotaim-barai</i>	33.7	HOM	SM	SM	SM	Small	7–8	8–9	129–138	23–25	72–79	117–121	–	5–6	2–4	3	16–17	19–20
<i>C. kawminiae sp. nov.</i>	35.6	HOM	SM	SM	SM	Small	7–8	7–8	107–114	17–21	76–78	86–92	–	7–8	2	4	14–15	15–16
<i>C. kumaras-inghei</i>	31.6	HOM	SM	SM	SM	Small	7–8	7–8	120–134	17–21	87–94	61–68	–	7–9	2–3	3–5	15–16	16–18
<i>C. latha</i>	30.4	HOM	SM	SM	SM	Small	7–8	7–8	109–115	13–15	69–73	72–79	–	5–7	2–3	4–5	15–17	17–18

Table 9 (continued). Key characters of 36 currently known *Cnemaspis* species in Sri Lanka. Abbreviations: mm – Millimeters, SVL – Maximum Snout to vent length, SUB – Subcaudals, SUP – Supralabials, INF – Infralabials, PG – Paravertebral granules, IFS – Interfemoral scales, FLSP – Flank spines, PCP – Precloacal pores, FP – Femoral pores, HET – Heterogeneous, HOM – Homogeneous, KD – Keeled, SM – Smooth.

<i>C. nandimithrai</i>	31.7	HOM	SM	SM	SM	SM	SM	Small	5–6	6	108–112	25–27	87–89	95–99	–	3–4	2–4	2–4	12–13	19–20
Clade (2) <i>Cnemaspis podihuna</i> (subcaudals large)																				
Group (1) – <i>scalpensis</i>																				
<i>C. alwisi</i>	40.4	HOM	SM	SM	SM	SM	SM	Enlarged	8–10	7–9	145–153	27–31	71–78	89–97	18–19	4–5	–	7–9	15–17	17–21
<i>C. anslemi</i>	34.4	HOM	SM	SM	SM	SM	SM	Enlarged	8–9	8–9	111–117	19–21	87–91	118–122	9–10	3–4	–	14–15	16–17	20–21
<i>C. godagedarai</i>	35.5	HOM	SM	SM	SM	SM	SM	Enlarged	7–8	7–8	133–137	21–23	98–102	101–106	8	5–6	–	12–13	17–18	20–21
<i>C. gemunu</i>	34.0	HOM	SM	SM	SM	SM	SM	Enlarged	8–10	7–8	112–118	13–16	74–87	79–93	10–12	7–8	–	11–14	15–17	18–19
<i>C. hitihami</i>	41.7	HOM	SM	SM	SM	SM	SM	Enlarged	8–9	7–9	132–135	21	96–99	143–149	24–26	4–5	–	5–10	18–19	21–22
<i>C. kohukumburai</i>	34.5	HOM	SM	SM	SM	SM	SM	Enlarged	8–9	7–8	131–134	23	81–88	150–159	25	7–8	–	6–9	21–22	23–25
<i>C. nilgala</i>	32.9	HOM	SM	SM	SM	SM	SM	Enlarged	7–8	6–7	122–129	17–19	71–78	179–187	14–15	3–4	–	7–9	17	17–18
<i>C. philipsi</i>	36.6	HOM	SM	SM	SM	SM	SM	Enlarged	8–9	8–9	128–143	18–25	76–91	86–93	11–14	4–6	–	15–16	16–19	17–19
<i>C. punctata</i>	39.9	HOM	SM	SM	SM	SM	SM	Enlarged	7–10	7–9	129–137	20–29	71–78	83–91	25–27	11–13	–	5–7	17–18	17–23
<i>C. rajakarunai</i>	40.2	HOM	SM	SM	SM	SM	SM	Enlarged	8–9	9–11	146–186	26–29	69–74	81–85	20–22	5–6	–	7–8	16–20	19–22
<i>C. rammalensis</i>	53.8	HOM	SM	SM	SM	SM	SM	Enlarged	8–10	8–9	186–207	25–28	119–131	94–96	19–24	4–5	–	14–16	22–23	22–23
<i>C. scalpensis</i>	36.6	HOM	SM	SM	SM	SM	SM	Enlarged	7–9	7–8	120–131	17–19	81–89	102–112	8–12	9–11	–	13–15	17–18	19–21
Group (2) – <i>podihuna</i>																				
<i>C. kandambyi</i>	23.6	HOM	SM	SM	SM	SM	SM	Enlarged	7–8	7–8	128–137	16–17	71–77	85–92	–	4–5	3–4	5–6	11–14	19–20
<i>C. molligodai</i>	29.0	HOM	SM	SM	SM	SM	SM	Enlarged	8–10	7–8	127–135	15–19	73–82	76–83	–	5–7	4–5	8–9	15–18	19–23
<i>C. podihuna</i>	24.7	HOM	SM	SM	SM	SM	SM	Enlarged	7–9	6–8	111–118	15–19	79–83	102–106	–	4–6	3–4	3–6	14–15	18–19

tion of these new species are comparable to other congeners, which imply niche conservatism among divergent lineages.

The bulk of these rupicolous geckos are restricted to cool, moist, shady granite caves and rock walls or under granite boulders. According to our findings these geckos prefer narrow (~3–4 mm), long (~100–400 mm), and deep (~20–180 mm) crevices as refugia and oviposition sites. In several surveyed locations with granite caves, we were unable to find *Cnemaspis* species due to the lack of tall shady trees and adequately cool temperatures (substrate temperature: 25.2–28.7°C). A majority (66.7%) of *Cnemaspis* species are restricted to the wet bioclimatic zones of Sri Lanka and are point-endemic microhabitat specialists where distribution ranges are limited to <100 km². The restricted distribution could be an artifact of the limited availability of caves and similar microenvironments with granite or rock-based substrates. The high species richness in Sri Lanka may be accounted by the possibility of multiple, independent colonization events from the Indian mainland with subsequent, geographically-isolated *in-situ* speciation. The majority of the Indian *Cnemaspis* species have not been comparatively analyzed alongside the Sri Lankan species (Agarwal et al. 2017; Karunarathna et al. 2019b). The three new species described here are recorded from isolated locations in wet, intermediate, and dry bioclimatic zones of Sri Lanka. Of these, *C. kawminiae* **sp. nov.** is described from the wet zone montane region; *C. kotagamai* **sp. nov.** is described from the intermediate zone lowland; and *C. dissanayakai* **sp. nov.** is described from the dry zone lowland (Table 10). The record of *C. kawminiae* **sp. nov.** from Mandaramnuwara is noteworthy, as this is a high-altitude location nestled in the central highlands (1,400–1,800 m



Fig. 12. Threats to the isolated hill forests and *Cnemaspis* species in study areas in Sri Lanka. (A) Illegal forest clearing, (B) firewood collection for tea factory, (C) granite mining activities, (D) agricultural fields in slopy areas, (E) tea plantation and highly crowded anthropogenic habitat, and (F) a landslide in mountain areas. *Photos: Suranjan Karunaratna and Madhava Botejue.*

asl), making this the 4th species in the genus found at an elevation above 1,000 m asl.

Bambaragala and Dimbulagala are isolated residual mountains and rock outcrops embedded within a forest and features granite caves incorporated with historical Buddhist monasteries, whereas Mandaramnuwara is a mixture of forested areas and rural human habitation. All these habitats are susceptible to human-induced habitat degradation, including clear cutting and timber felling, forest fragmentation, granite mining, tea cultivation (Fig. 12), rubber cultivation, vegetable farming, invasive species, human settlements, road and other infrastructure development, and waste disposal (see Karunaratna et

al. 2017). Bambaragala, situated in Ratnapura District of Sabaragamuwa Province, is the most vulnerable habitat as it is a small forested rock outcrop (~50 ha) located amidst a rapidly urbanizing landscape; where a part of the rock outcrop is currently undergoing mining, making *C. kotagamai* **sp. nov.** the most endangered amongst these new species. However, many such habitats are somewhat protected due to the presence of Buddhist monasteries which serve as refugia for reptiles and other faunal groups, and it is imperative to conserve these habitats to protect the island's unique biodiversity (Amarasinghe et al. 2016; Edirisinghe et al. 2018; Karunaratna et al. 2019a). Sri Lanka's tropical humid wet zone is

Table 10. Distribution and ecological data of the three new *Cnemaspis* species from Sri Lanka. Abbreviations: m – meters; ha – hectares; mm – millimeters; Lux – light intensity; CR – Critically Endangered.

Species	Bioclimatic area	District	Coordinates		Elevation	Area size	Forest type	Microhabitat	Rainfall (mm)	Annual temperature	Ambient temperature	Substrate temperature	Light intensity (Lux)	Relative humidity	Canopy cover	Conservation status (IUCN)
			N	E												
<i>C. kotagamai</i> sp. nov.	Intermediate zone	Ratnapura	6.512978	80.750306	127 m	50 ha	Semievergreen	Granite cave	1,500–2,000	27.8–29.6 °C	29.8 °C	28.1 °C	469	83%	80%	CR
			6.517261	80.752692	132 m			Granite cave			30.6 °C	28.6 °C	385	88%	65%	
			6.520786	80.748225	122 m			Old building			29.6 °C	27.8 °C	448	79%	65%	
			6.510536	80.746783	139 m			Granite cave			31.3 °C	28.4 °C	455	71%	70%	
<i>C. dissanayakai</i> sp. nov.	Dry zone	Polonnaruwa	7.872931	81.135569	129 m	1,000 ha	Dry mixed evergreen	Granite cave	1,500–2,000	28.9–30.2 °C	31.4 °C	27.9 °C	648	85%	70%	CR
			7.851358	81.141675	135 m			Granite cave			30.7 °C	28.2 °C	594	75%	75%	
			7.860200	81.114836	126 m			Old building			30.2 °C	28.6 °C	617	80%	65%	
			7.850547	81.127831	131 m			Old building			31.9 °C	27.5 °C	625	70%	90%	
<i>C. kawmini</i> sp. nov.	Wet zone	Nuwara-Eliya	7.033558	80.798794	1,600 m	300 ha	Wet evergreen	Granite wall	3,000–4,000	27.4–28.9 °C	25.8 °C	27.9 °C	592	74%	70%	CR
			7.020600	80.788639	1,658 m			Granite cave			26.5 °C	26.7 °C	566	83%	75%	
			7.034775	80.783497	1,574 m			Granite wall			26.1 °C	27.5 °C	486	92%	85%	
			7.028314	80.773903	1,592 m			Granite wall			24.2 °C	28.3 °C	578	87%	80%	

globally recognized for its exceptionally high biodiversity and endemism (Bossuyt et al. 2004). Nonetheless, the new species reported here and in previous studies on the same genus continue to illustrate the undocumented diversity of *Cnemaspis* that also occurs within the dry and intermediate bioclimatic zones (Batuwita et al. 2019; Karunarathna et al. 2019b).

Most of the *Cnemaspis* species from the dry and intermediate climatic zones of Sri Lanka are, however, restricted to small isolated habitats scattered over the lowlands (Batuwita et al. 2019; Karunarathna et al. 2019a,b). The presence of granitic caves and the humid forest cover surrounding the caves seem to serve as ideal refugia for these geckos with narrow, specialized ecological niches. It is very likely that future studies on the biogeography of *Cnemaspis* in Sri Lanka will highlight the importance of these isolated habitats in generating and maintaining the diversity of these unique groups of geckos in the island (Karunarathna and Amarasinghe 2011; Amarasinghe et al. 2016). At the same time, it is important to note that the point endemic species described here, which are highly sensitive to changes in the habitat, would be severely affected by habitat degradation. Hence, past and present studies have emphasized the importance of conserving such isolated habitats throughout the country (Karunarathna and Amarasinghe 2013; Gabadage et al. 2018). Though traditional conservation strategies usually target extensive natural habitats to maximize biodiversity conservation, our studies indicate that these small isolated habitats also deserve the immediate attention of conservation authorities. Thus, we believe our findings on these geckos and their granite cave and rock-associated habitats add a new dimension to the biodiversity conservation of Sri Lanka.

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Literature Cited

- Agarwal I, Biswas S, Bauer AM, Greenbaum E, Jackman TR, de Silva A, Batuwita S. 2017. Cryptic species, taxonomic inflation, or a bit of both? New species phenomenon in Sri Lanka as suggested by a phylogeny of dwarf geckos (Reptilia, Squamata, Gekkonidae, *Cnemaspis*). *Systematics and Biodiversity* 15: 1–13.
- Amarasinghe T, Campbell P, Madawala M, Botejue M, Gabadage D, de Silva A, Karunarathna S. 2016. The re-discovery of the live populations of *Cnemaspis tropidogaster* (Boulenger, 1885) (Sauria: Gekkonidae) from Sri Lanka after 120 years. *Zootaxa* 4200: 395–405.
- Batuwita S, Udugampala S. 2017. Description of a new species of *Cnemaspis* (Squamata: Gekkonidae) from Knuckles Range of Sri Lanka. *Zootaxa* 4254: 82–90.
- Batuwita S, Agarwal I, Bauer AM. 2019. Description of a new diminutive, rupicolous species of day-gecko (Squamata: Gekkonidae: *Cnemaspis*) from southern Sri Lanka. *Zootaxa* 4565: 223–234.
- Bauer AM, de Silva A, Greenbaum E, Jackman T. 2007. A new species of day gecko from high elevation in Sri Lanka, with a preliminary phylogeny of Sri Lankan *Cnemaspis* (Reptilia: Squamata: Gekkonidae). *Mitteilungen aus dem Museum für Naturkunde, Berlin, Zoologische Reihe* 83: 22–32.
- Bauer AM, Jackman TR, Greenbaum E, Giri VB, de Silva A. 2010. South Asia supports a major endemic radiation of *Hemidactylus* geckos. *Molecular Phylogenetics & Evolution* 57: 343–352.
- Bossuyt F, Meegaskumbura M, Beenaerts N, Gower DJ, Pethiyagoda R, Roelants K, Mannaert A, Wilkinson M, Bahir MM, Manamendra-Arachchi K, et al. 2004. Local endemism within the Western Ghats-Sri Lanka biodiversity hotspot. *Science* 306: 479–481.
- Boulenger GA. 1885. *Catalogue of the Lizards in the British Museum (Natural History)*. British Museum of Natural History, London, United Kingdom. 436 p.
- Das I. 2005. Revision of the genus *Cnemaspis* Strauch, 1887 (Sauria: Gekkonidae), from the Mentawai and adjacent archipelagos off Western Sumatra, Indonesia, with the description of four new species. *Journal of Herpetology* 39: 233–247.
- Deraniyagala PEP. 1944. A new *Cnemaspis* gecko from Ceylon. *Journal of Royal Asiatic Society Sri Lanka* 97: 226–227.
- Deraniyagala PEP. 1953. *A Colored Atlas of Some Vertebrates from Ceylon (Tetrapoda Reptilia)*. Volume 2. National Museums of Sri Lanka, Colombo, Sri Lanka. 101 p.
- de Silva A, Bauer AM, Botejue M, Karunarathna S. 2019. A new species of endemic day gecko (Reptilia: Gekkonidae: *Cnemaspis*) from a wet zone forest in second peneplain of Southern Sri Lanka. *Amphibian & Reptile Conservation* 13(1) [General Section]: 198–208 (e177).
- Edirisinghe G, Surasinghe T, Gabadage D, Botejue M, Perera K, Madawala M, Weerakoon D, Karunarathna S. 2018. Chiropteran diversity in the peripheral areas of the Maduru-Oya National Park in Sri Lanka: insights for conservation and management. *ZooKeys* 784: 139–162.
- Ferguson WM. 1877. *Reptile Fauna of Ceylon. Letter on a Collection Sent to the Colombo Museum*. William Henry Herbert, Government Printer, Colombo, Ceylon (Sri Lanka). 42 p.
- Gabadage D, Surasinghe T, de Silva A, Somaweera R, Madurapperuma B, Madawala M, Botejue M, Karunarathna S. 2018. Ecological and zoological study of endemic Sri Lankan Keelback (*Balanophis ceylonensis*): with implications for its conservation. *Vertebrate Zoology* 68: 225–236.
- Gamble T, Greenbaum E, Jackman TR, Russell AP, Bauer AM. 2012. Repeated origin and loss of adhesive toepads in geckos. *PLoS One* 7: e39429.
- Grismer LL, Wood PL, Anuar S, Riyanto A, Ahmad N, Muin MA, Sumontha M, Grismer JL, Onn CK, Quah ESH, et al. 2014. Systematics and natural history of Southeast Asian rock geckos (genus *Cnemaspis* Strauch, 1887) with descriptions of eight new species from Malaysia, Thailand, and Indonesia. *Zootaxa* 3880: 1–147.
- Gunatileke IAUN, Gunatileke CVS. 1990. Distribution of floristic richness and its conservation in Sri Lanka. *Conservation Biology* 4: 21–31.
- Hammer O, Harper DAT, Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4: 1–9.
- Iskandar DT, McGuire JA, Amarasinghe AAT. 2017. Description of five new day geckos of *Cnemaspis kandiana* group (Sauria: Gekkonidae) from Sumatra and Mentawai Archipelago, Indonesia. *Journal of Herpetology* 51: 142–153.
- Karunarathna S, Amarasinghe T. 2011. Natural history and conservation status of *Calodactylodes illingworthorum* Deraniyagala, 1953 (Sauria: Gekkonidae) in south-eastern Sri Lanka. *Herpetotropicos* 6: 5–10.
- Karunarathna S, Amarasinghe T. 2013. Behavioral ecology and microhabitat use by *Lyriocephalus scutatus* (Linnaeus, 1758): a monotypic genus in Sri Lanka (Reptilia: Agamidae: Draconinae) with notes on the taxonomy. *Russian Journal of Herpetology* 20: 1–15.
- Karunarathna S, Henkanaththegedara S, Gabadage D, Botejue M, Madawala M, Surasinghe T. 2017. Ecology and demography of the Critically Endangered Kandian Torrent Toad *Adenomus kandianus*: a long-lost endemic species of Sri Lanka. *Oryx* 51: 619–626.

- Karunarathna S, Bauer A, de Silva A, Surasinghe T, Somaratna L, Madawala M, Gabadage D, Botejue M, Henkanaththegedara S, Ukuwela K. 2019a. Description of a new species of the genus *Cnemaspis* Strauch, 1887 (Reptilia: Squamata: Gekkonidae) from the Nilgala Savannah forest, Uva Province of Sri Lanka. *Zootaxa* 4545: 389–407.
- Karunarathna S, Poyarkov NA, de Silva A, Madawala M, Botejue M, Gorin VA, Surasinghe T, Gabadage D, Ukuwela KDB, Bauer AM. 2019b. Integrative taxonomy reveals six new species of day geckos of the genus *Cnemaspis* Strauch, 1887 (Reptilia: Squamata: Gekkonidae) from geographically isolated hill forests in Sri Lanka. *Vertebrate Zoology* 64: 247–298.
- Karunarathna S, Ukuwela K. 2019. A new species of dwarf day gecko (Reptilia: Gekkonidae: *Cnemaspis*) from lower-elevations of Samanala Nature Reserve in Central massif, Sri Lanka. *Amphibian & Reptile Conservation* 13(2) [General Section]: 14–27 (e187).
- Kelaart EF. 1852. *Prodromus Faunae Zeylanicae, being Contributions to the Zoology of Ceylon*. Volume 1. Published by the author, Colombo, Sri Lanka. 197 p.
- Manamendra-Arachchi K, Batuwita S, Pethiyagoda R. 2007. A taxonomic revision of the Sri Lankan day geckos (Reptilia: Gekkonidae: *Cnemaspis*), with description of new species from Sri Lanka and Southern India. *Zeylanica* 7: 9–122.
- Meegaskumbura M, Senevirathne G, Manamendra-Arachchi K, Pethiyagoda R, Hanken J, Schneider CJ. 2019. Diversification of shrub frogs (Rhacophoridae: *Pseudophilautus*) in Sri Lanka—Timing and geographic context. *Molecular Phylogenetics & Evolution* 132: 14–24.
- Pyron RA, Burbrink FT, Wiens JJ. 2013a. A phylogeny and revised classification of Squamata, including 4,161 species of lizards and snakes. *BMC Evolutionary Biology* 13: 1–53.
- Pyron RA, Kandambi HKD, Hendry CR, Pushpamal V, Burbrink FT, Somaweera R. 2013b. Genus-level phylogeny of snakes reveals the origins of species richness in Sri Lanka. *Molecular Phylogenetics & Evolution* 66: 969–978.
- R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available: <https://www.R-project.org/> [Accessed: 15 July 2019].
- Riyanto A, Hamidy A, Sidik I, Gunalen D. 2017. A new species of Rock Gecko of the genus *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from Belitung Island, Indonesia. *Zootaxa* 4358: 583–597.
- RStudio Team. 2018. RStudio: integrated development for R. RStudio, Inc., Boston, Massachusetts, USA. Available: <http://www.rstudio.com/> [Accessed: 26 September 2019].
- Stacklies W, Redestig H, Scholz, M, Walther D, Selbig J. 2007. pcaMethods—a bioconductor package providing PCA methods for incomplete data. *Bioinformatics* 23: 1,164–1,167.
- Uetz P, Freed P, Hošek J. 2019. The Reptile Database. Available: <http://reptile-database.reptarium.cz/> [Accessed: 20 December 2019].
- Uetz P, Cherikh S, Shea G, Ineich I, Campbell PD, Doronin IV, Rosado J, Wynn A, Tighe KA, McDiarmid R, et al. 2019b. A global catalog of primary reptile type specimens. *Zootaxa* 4695: 438–450.
- Vidanapathirana DR, Rajeev MDG, Wickramasinghe N, Fernando SS, Wickramasinghe LJM. 2014. *Cnemaspis rammalensis* sp. nov., Sri Lanka’s largest day-gecko (Sauria: Gekkonidae: *Cnemaspis*) from Rammalakanda Man and Biosphere Reserve in southern Sri Lanka. *Zootaxa* 3755: 273–286.
- Wickham H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York, New York, USA. 259 p.
- Wickramasinghe LJM, Munindradasa DAI. 2007. Review of the genus *Cnemaspis* Strauch, 1887 (Sauria: Gekkonidae) in Sri Lanka with the description of five new species. *Zootaxa* 1490: 1–63.
- Wickramasinghe LJM, Vidanapathirana DR, Rathnayake RMGP. 2016. *Cnemaspis rajakarunai* sp. nov., a rock dwelling day-gecko (Sauria: Gekkonidae: *Cnemaspis*) from Salgala, an unprotected lowland rainforest in Sri Lanka. *Zootaxa* 4168: 92–108.
- Wood PL, Grismer LL, Aowphol A, Aguilar CA, Cota M, Grismer MS, Murdoch ML, Sites JW Jr. 2017. Three new karst-dwelling *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from Peninsular Thailand and the phylogenetic placement of *C. punctatomuchalis* and *C. vandeventeri*. *Peer J* 5: e2884.
- Zheng Y, Wiens JJ. 2016. Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4,162 species. *Molecular Phylogenetics & Evolution* 94: 537–547.



Suranjan Karunarathna began his scientific exploration of biodiversity with the Young Zoologists’ Association of Sri Lanka (YZA) in early 2000, and led the society in 2007 as the President. Suranjan earned his Masters of Environmental Management from University of Colombo, Sri Lanka, in 2017. As a wildlife researcher, he studies herpetofaunal ecology and taxonomy, and also promotes science-based conservation awareness on the importance of biodiversity and its conservation among the Sri Lankan community. Suranjan is an active member of several specialist groups of IUCN/SSC, and has served as an expert committee member of the IUCN Global and National Red List development programs since 2004.



Anselm de Silva M.Sc., D.Sc. (University of Peradeniya, Sri Lanka) started keeping reptiles at the early age of seven, and he has taught herpetology at the Rajarata University of Sri Lanka and mentored final-year veterinary students at University of Peradeniya. Anselm has conducted herpetofaunal surveys in most of the important ecosystems in the country, and has published more than 400 papers, of which nearly 60 are books or book chapters. Anselm had done yeoman service to the country and the region for more than 50 years. He is the Regional Chairman of the Crocodile Specialist Group for South Asia and Iran, Co-Chair of the Amphibian Specialist Group IUCN/SSC Sri Lanka. Anselm received the IUCN/SSC Sir Peter Scott Award for Conservation Merit in October 2019 – the first Sri Lankan to receive this prestigious award.



Madhava Botejue has been engaged in research on the biodiversity, ecology, distribution, behavior, taxonomy, and conservation of Sri Lankan fauna for the past 14 years, with a main focus on herpetofauna, avifauna, and mammals. Madhava has contributed to environmental protection through many community-based awareness programs on the importance of biodiversity and its conservation. He earned his B.Sc. degree in Natural Sciences from The Open University of Sri Lanka in 2009. Currently, he serves as an Environmental Officer at the Central Environmental Authority, Sri Lanka, a member of IUCN/SSC Crocodile Specialist Group, and an expert committee member of IUCN Global and National Red List development programs.



Dinesh Gabadage is a field biologist who began his wildlife interests in 1990 as a member of the Young Zoologists Association of Sri Lanka (YZA), and also in 1994 as a member of the Wildlife Heritage Trust of Sri Lanka (WHT). Dinesh is a dedicated researcher studying the biodiversity ecology, distribution, behavior, and taxonomy of herpetofauna, avifauna, and mammals in Sri Lanka; and he has conducted many community-based programs promoting wildlife conservation. He is also an expert committee member in the IUCN Global and National Red List development programs, and earned his Diplomas in Palaeo-biodiversity and Zooarchaeology from University of Kelaniya, Sri Lanka.



Lankani Somaratne, B.Sc. (University of Colombo, Sri Lanka), is a zoologist who started her career as an Assistant Director in the Zoology Division of the Department of National Museums five years ago. Lankani has engaged in re-cataloging and updating of the avifaunal, skink, amphibian, and ichthyological collections at the National Museum for the past five years. She has contributed to enhancing the knowledge on the museological aspects of Natural Specimen conservation for different communities. She is a member of the International Community of Museums (ICOM), representing Sri Lanka. Apart from zoological conservation, she is currently working on conservation project at the Dutch Museum, Sri Lanka.



Angelo Hettige began his interest in wildlife from a very young age. His interests began to grow as a member of the Young Zoologists Association of Sri Lanka (YZA) since the early 2000s, from the junior group continuing up to the senior group. Angelo has contributed to conservation through community awareness programs on the importance of reptiles and their conservation, and through numerous snake rescues. Currently, he is working in the snake anti-venom research project at the University of Peradeniya, Sri Lanka, and he wishes to continue his career studying herpetofauna and its conservation.



Nimantha Aberathna is a naturalist who began his career and wildlife interests in 2004 as a naturalist, and as a member of the Youth Exploration Society of Sri Lanka (YES) in 2009. He served as the President of the Research and Education Committee during 2015-2017. Nimantha holds a certificate of Wildlife Conservation and Management from the Open University of Sri Lanka. As a wildlife researcher, he is studying ichthyofauna and orchid ecology and taxonomy. He is also engaged in a captive breeding program for threatened species, and has been involved in many snake rescue events. Nimantha worked as a venom extractor for the snake anti-venom research project at the University of Peradeniya, Sri Lanka.



Majintha Madawala is a naturalist who began his career and wildlife interests in 1995 as a member of the Young Zoologists Association of Sri Lanka (YZA), and holds a Diploma in Biodiversity Management and Conservation from the University of Colombo, Sri Lanka. As a conservationist and a naturalist, he is engaged in numerous habitat restoration, snake rescue programs, and biodiversity research projects in Sri Lanka. Currently, Majintha is engaged in herpetofaunal research with the Victorian Herpetological Society in Australia. He is also an active member of the IUCN/SSC Crocodile Specialist Group and IUCN Global and National Red List development programs.

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Gayan Edirisinghe began his studies on wildlife in 2000 when he became a member of the Young Zoologists Association of Sri Lanka (YZA), which laid a strong foundation for his interest in mammals. Gayan initiated his research career in 2005 with a study on small mammals. For the past ten years, he has been involved in many research projects on Sri Lankan fauna, mainly focusing on the diversity, distribution, ecology, behavior, and conservation of chiropterans. He has conducted awareness programs to educate the community on the importance of biodiversity and its conservation, and earned his Diplomas in Palaeo-biodiversity and Zoo-archaeology from University of Kelaniya, Sri Lanka.



Nirmala Perera has been a member of Young Zoologists' Association (YZA) since 1999, and he has conducted several awareness programs on biodiversity conservation through many different levels of the society. Nirmala also served as the secretary of the action committee of YZA, and he has worked actively on several environmental issues raised in Sri Lanka. He holds a Diploma in Biodiversity Management from the University of Colombo and worked as the snake biologist in the snake venom research project in the Faculty of Medicine, University of Colombo. He also worked as a Project Coordinator at Udawalawe Human-Elephant Conflict Program of the Born Free Foundation, Sri Lanka (2011–2014).



Sulakshana Wickramaarachchi is a hardware and software engineer by profession, but began his studies on wildlife conservation in 2006 as a member of the Young Zoologists' Association (YZA), and later served as a committee member of the Research Committee and as the Treasurer during 2011–2012. He is also engaged in captive breeding programs for threatened species and many snakes rescue missions. He also conducts awareness programs to promote the importance of snake fauna and its conservation among the Sri Lankan community. Also he worked as an assistant venom extractor of the snake venom research project in the Faculty of Medicine, University of Colombo.



Thilina Surasinghe is an Assistant Professor in the Department of Biological Sciences in Bridgewater State University, Massachusetts, USA, and obtained his Ph.D. in Wildlife Biology at Clemson University, South Carolina, USA. Thilina is an ecologist; his academic training encompasses different aspects of biology, ecology, environmental sciences, and natural resources management. He is experienced in teaching undergraduates in biology, environmental sciences, and social sciences; and he takes part in projects on landscape-scale biodiversity assessments, Red List assessments, conservation planning, GIS based threat and GAP analyses, and EPA protocols.



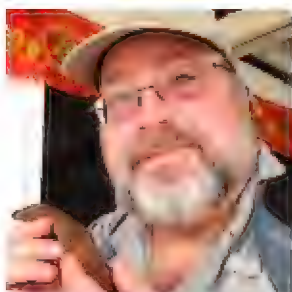
Niranjana Karunarathna is a naturalist who loves traveling, camping, and hiking. He has been a member of Young Zoologists' Association (YZA) since 2006, has participated in many herpetological research projects and also has ongoing funded projects. Niranjana is also conducting wildlife photography, biodiversity conservation, and educational programs for the Sri Lankan community.



Mendis Wickramasinghe founded the Herpetological Foundation of Sri Lanka (HFS), to further pursue independent research on the herpetofauna of Sri Lanka, while providing a platform for young herpetologists to initiate research. With nearly 25 years of field research experience on the herpetofauna of Sri Lanka, his work has focused on taxonomic identification and biodiversity assessments of amphibians and reptiles, in an effort to increase awareness on the importance of conserving their habitats in Sri Lanka. As a result, he has been able to discover and describe 29 new species of amphibians and reptiles, and participated in the re-discovery of three “extinct” amphibian species.



Kanishka D.B. Ukuwela is currently a Senior Lecturer in Zoology at the Rajarata University of Sri Lanka. He holds a B.Sc. (Hons.) degree in Zoology from the University of Peradeniya, Sri Lanka and a Ph.D. in Evolutionary Biology from the University of Adelaide, Australia. His current research focuses on the origins, evolution, systematics, and conservation of the South Asian herpetofauna.



Aaron Bauer grew up collecting reptiles and amphibians in his native New York. He is the Gerald M. Lemole Endowed Professor of Integrative Biology at Villanova University in Pennsylvania, USA, and has been studying reptiles, especially geckos, for more than 35 years. Aaron has worked widely in Sri Lanka, India, southern Africa, Australia, and the South Pacific; and has described nearly 200 species of reptiles and written more than 750 publications. He is a former Secretary General of the World Congress of Herpetology, President of the Society for the Study of Amphibians and Reptiles, President of the Herpetologists' League, and Chairman of the Herpetological Association of Africa.

Appendix 1.

Comparative *Cnemaspis* materials examined from Sri Lanka

Cnemaspis alwisi: NMSL 2004.09.01 (holotype), NMSL 2004.09.02 (paratype), NMSL 2004.09.03 (paratype), WHT 5918, WHT 6518, WHT 6519, WHT 7336, WHT 7337, WHT 7338, WHT 7343, WHT 7344, WHT 7345, WHT 7346.

C. anslemi: NMSL 2019.14.01 (holotype), NMSL 2019.14.02 (paratype), NMSL 2019.14.03 (paratype).

C. amith: BMNH 63.3.19.1066A (holotype), BMNH 63.3.19.1066B (paratype), BMNH 63.3.19.1066C (paratype).

C. butewai: NMSL 2019.07.01 (holotype), NMSL 2019.07.02 (paratype), NMSL 2019.07.03 (paratype).

C. gemunu: AMB 7495 (holotype), AMB 7507 (paratype?), WHT 7221, WHT 7347, WHT 7348, NMSL 2006.11.01, NMSL 2006.11.02, NMSL 2006.11.03, NMSL 2006.11.04.

C. godagedarai: NMSL 2019.09.01 (holotype), NMSL 2019.16.01 (paratype), NMSL 2019.16.02 (paratype).

C. gotaimbarai: NMSL 2019.04.01 (holotype), NMSL 2019.04.02 (paratype), NMSL 2019.04.03 (paratype).

C. hitihami: NMSL 2019.06.01 (holotype), NMSL 2019.06.02 (paratype), NMSL 2019.06.03 (paratype).

C. ingerorum: WHT 7332 (holotype), WHT 7330 (paratype), WHT 7331 (paratype).

C. kallima: WHT 7245 (holotype), WHT 7222 (paratype), WHT 7227 (paratype), WHT 7228 (paratype), WHT 7229 (paratype), WHT 7230 (paratype), WHT 7239 (paratype), WHT 7249 (paratype), WHT 7251 (paratype), WHT 7252 (paratype), WHT 7253 (paratype), WHT 7254 (paratype), WHT 7255 (paratype).

C. kandambyi: WHT 9466 (holotype), WHT 9467 (paratype).

C. kandiana: BMNH 53.4.1.1 (lectotype), BMNH 80.2.2.119A (paralectotype), BMNH 80.2.2.119B (paralectotype), BMNH 80.2.2.119C (paralectotype), WHT 7212, WHT 7213, WHT 7267, WHT 7305, WHT 7307, WHT 7308, WHT 7310, WHT 7313, WHT 7319, WHT 7322.

C. kivulegedarai: NMSL 2019.08.01 (holotype), NMSL 2019.08.02 (paratype), NMSL 2019.08.03 (paratype).

C. kohukumburai: NMSL 2019.05.01 (holotype), NMSL 2019.05.02 (paratype), NMSL 2019.05.03 (paratype).

C. kumarasinghei: NMSL 2006.13.01 (holotype), NMSL 2006.13.02 (paratype).

C. latha: WHT 7214 (holotype).

C. menikay: WHT 7219 (holotype), WHT 7218 (paratype), WHT 7349 (paratype).

C. molligodai: NMSL 2006.14.01 (holotype), NMSL 2006.14.02 (paratype), NMSL 2006.14.03 (paratype), NMSL 2006.14.04 (paratype), NMSL 2006.14.05 (paratype).

C. nandimithrai: NMSL 2019.01.01 (holotype), NMSL 2019.01.02 (paratype), NMSL 2019.01.03 (paratype).

C. nilgala: NMSL 2018.07.01 (holotype), NMSL 2018.06.01 (paratype), NMSL 2018.06.02 (paratype), NMSL 2018.06.03 (paratype).

C. pava: WHT 7286 (holotype), WHT 7281 (paratype), WHT 7282 (paratype), WHT 7283 (paratype), WHT 7285 (paratype), WHT 7288 (paratype), WHT 7289 (paratype), WHT 7290 (paratype), WHT 7291 (paratype), WHT 7292 (paratype), WHT 7293 (paratype), WHT 7294 (paratype), WHT 7295 (paratype), WHT 7296 (paratype), WHT 7297 (paratype), WHT 7298 (paratype), WHT 7299 (paratype), WHT 7300 (paratype), WHT 7301 (paratype), WHT 7302 (paratype).

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C. phillipsi: WHT 7248 (holotype), WHT 7236 (paratype), WHT 7237 (paratype), WHT 7238 (paratype).

C. podihuna: BMNH 1946.8.1.20 (holotype), NMSL 2006.10.02, NMSL 2006.10.03, NMSL 2006.10.04.

C. pulchra: WHT 7023 (holotype), WHT 1573a (paratype), WHT 7011 (paratype), WHT 7021 (paratype), WHT 7022 (paratype).

C. punctata: WHT 7256 (holotype), WHT 7223 (paratype), WHT 7226 (paratype), WHT 7243 (paratype), WHT 7244 (paratype).

C. rajakarunai: NMSL 2016.07.01 (holotype), DWC 2016.05.01 (paratype), DWC 2016.05.02 (paratype).

C. rammalensis: NMSL 2013.25.01 (holotype), DWC 2013.05.001.

C. retigalensis: NMSL 2006.12.01 (holotype), NMSL 2006.12.02 (paratype), NMSL 2006.12.03 (paratype), NMSL 2006.12.04 (paratype).

C. samanaleensis: NMSL 2006.15.01 (holotype), NMSL 2006.15.02 (paratype), NMSL 2006.15.03 (paratype), NMSL 2006.15.04 (paratype), NMSL 2006.15.05 (paratype).

C. scalpensis: NMSL 2004.01.01 (neotype), NMSL 2004.02.01, NMSL 2004.03.01, NMSL 2004.04.01, WHT 7265, WHT 7268, WHT 7269, WHT 7274, WHT 7275, WHT 7276, WHT 7320.

C. silvula: WHT 7208 (holotype), WHT 7206 (paratype), WHT 7207 (paratype), WHT 7209 (paratype), WHT 7210 (paratype), WHT 7216 (paratype), WHT 7217 (paratype), WHT 7018, WHT 7027, WHT 7202, WHT 7203, WHT 7220, WHT 7354, WHT 7333.

C. tropidogater: BMNH 71.12.14.49 (lectotype), NMSL 5152, NMSL 5151, NMSL 5159, NMSL 5157, NMSL 5970, NMSL 5974.

C. upendrai: WHT 7189 (holotype), WHT 7184 (paratype), WHT 7187 (paratype), WHT 7188 (paratype), WHT 7181 (paratype), WHT 7182 (paratype), WHT 7183 (paratype), WHT 7185 (paratype), WHT 7190 (paratype), WHT 7191 (paratype), WHT 7192 (paratype), WHT 7193 (paratype), WHT 7194 (paratype), WHT 7195 (paratype), WHT 7196 (paratype), WHT 7197 (paratype), WHT 7260 (paratype).

Editorial

Manuscript reviewers for *Amphibian & Reptile Conservation* (2019)

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In 2019, ARC published 49 numbered items, including 45 peer-reviewed articles, 3 book reviews, and a collection of personal tributes to the late Bill Branch. We are very grateful to the peer-reviewers listed below for volunteering their time and expertise to help us determine which of the submitted manuscripts that passed our initial screening merited publication, and also for providing suggestions that improved the analysis, interpretation, and presentation of the data and ideas in each article. The following individuals have provided either manuscript reviews during 2019 or prior reviews of manuscripts that were published in 2019 (Volume 13). The names of those who reviewed more than one manuscript are given in bold. We look forward to the continuing generous gift of time from the reviewers who will help us maintain the high quality of articles published in ARC in the future.

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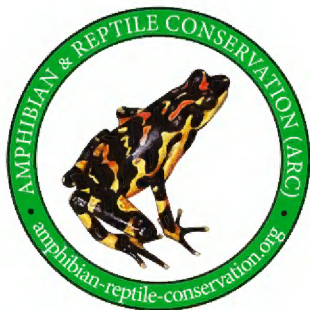
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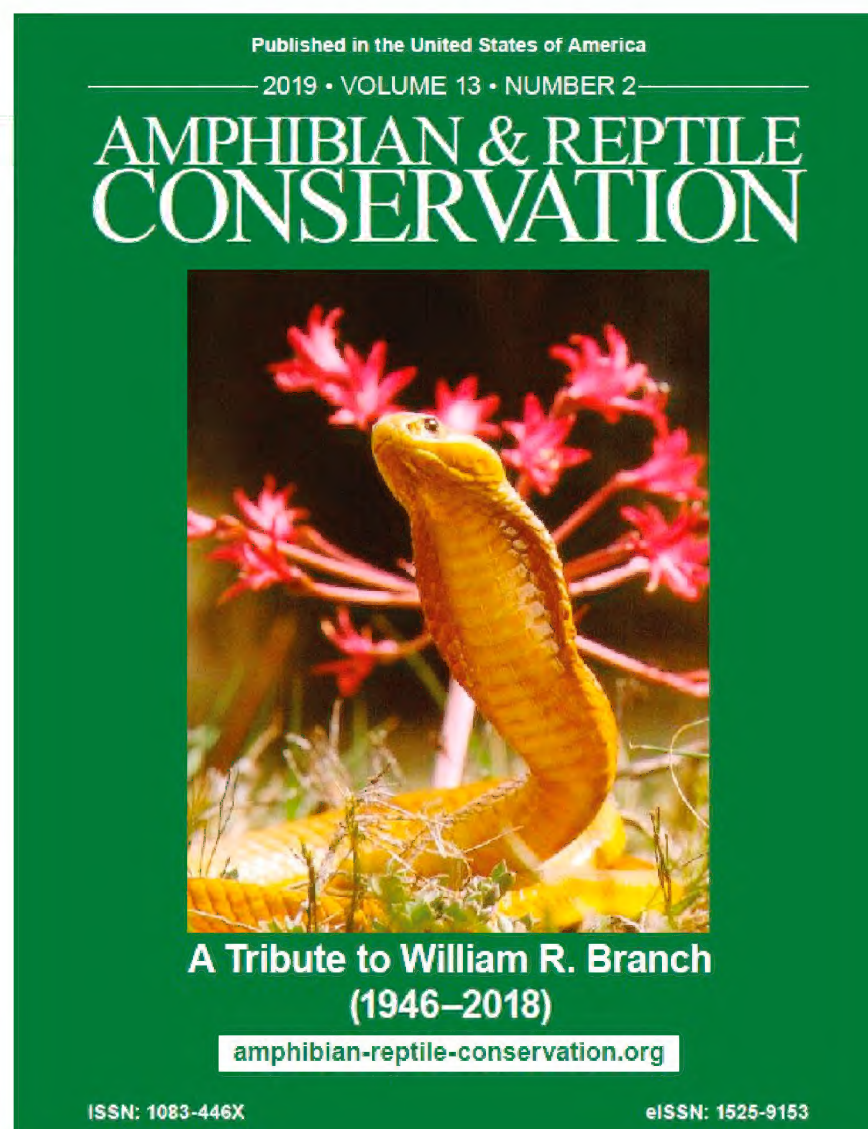
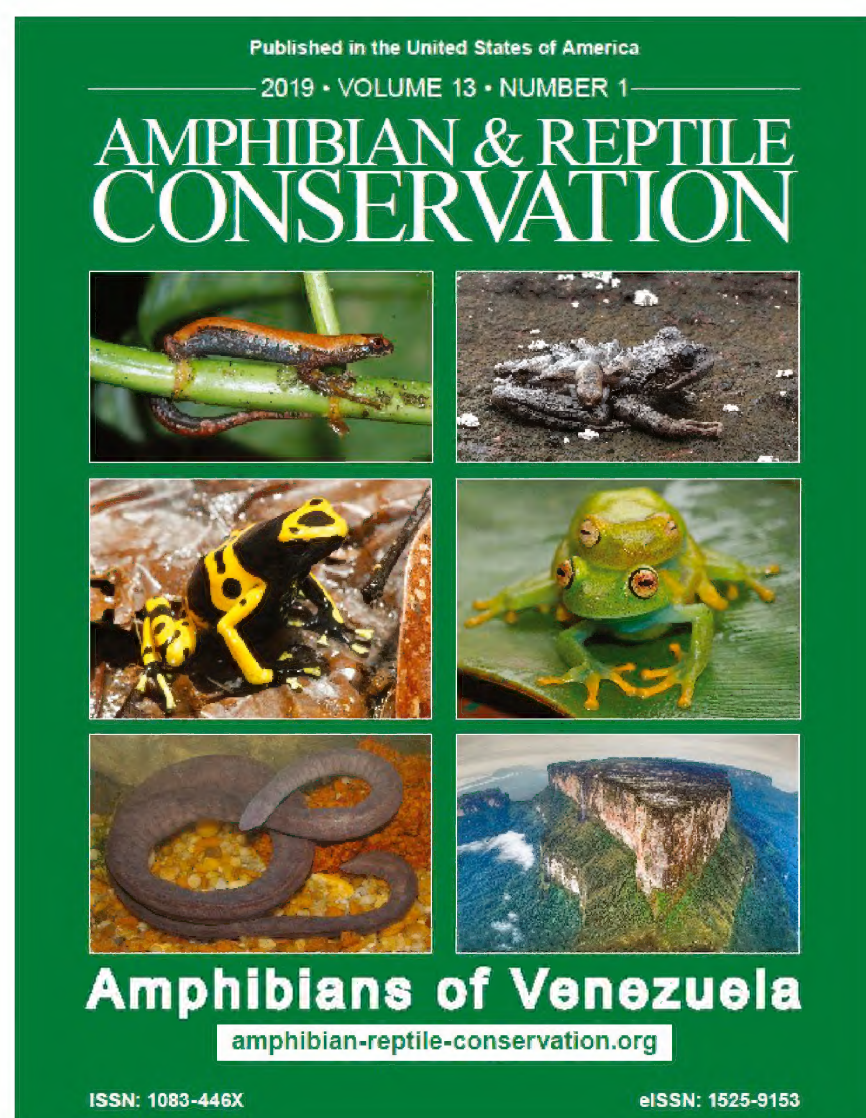
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